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February 26, 1999

Attorney Docket No.: 10287/043001

Box Patent Application

Assistant Commissioner for Patents
Washington, DC 20231

Presented for filing is a new patent application claiming priority from a provisional patent application of:

Applicant: KATIA GEORGOPOULOS, BRUCE A. MORGAN, CLAIRE KELLY
Title: THE HELIOS GENE

Enclosed are the following papers, including those required to receive a filing date under 37 CFR §1.53(b):

| | <u>Pages</u> |
|--------------------|-------------------------------|
| Specification | 73 |
| Claims | 2 |
| Abstract | 1 |
| Signed Declaration | [To Be Filed At A Later Date] |
| Drawing(s) | 14 |

Enclosures:

- Nucleotide and/or amino acid sequence listing including:
 - Computer readable copy.
 - Paper copy, pages.
 - Verification statement, 1 pages.
- Postcard.

"EXPRESS MAIL" Mailing Label Number ELO1008181705

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I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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Page 2

Under 35 USC §119(e)(1), this application claims the benefit of prior U.S. provisional application 60/076,325, filed February 27, 1998.

| | |
|---|------------|
| Basic filing fee | 760.00 |
| Total claims in excess of 20 times \$18.00 | 0.00 |
| Independent claims in excess of 3 times \$78.00 | 312.00 |
| Fee for multiple dependent claims | 260.00 |
| Total filing fee: | \$ 1332.00 |

A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

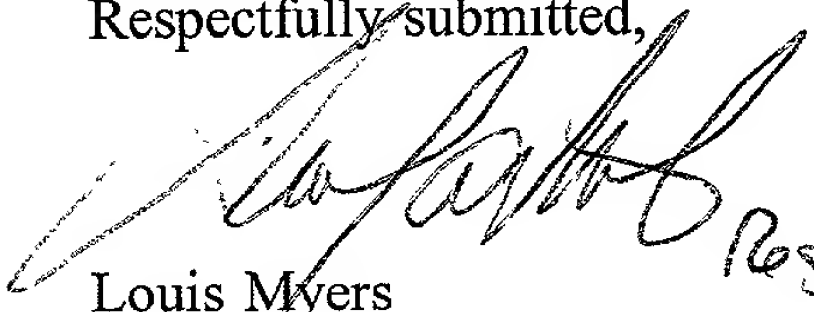
If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at 617/542-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

Please send all correspondence to:

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Respectfully submitted,


Louis Myers
Reg. No. 35,965
Enclosures

Reg No 30,175

**APPLICATION
FOR
UNITED STATES LETTERS PATENT**

TITLE: THE HELIOS GENE

APPLICANT: KATIA GEORGOPOULOS
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CLAIRE KELLY

"EXPRESS MAIL" Mailing Label Number ELD10081817US

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02/26/99

| Year | 1950 | 1951 | 1952 | 1953 | 1954 | 1955 | 1956 | 1957 | 1958 | 1959 | 1960 | 1961 | 1962 | 1963 | 1964 | 1965 | 1966 | 1967 | 1968 | 1969 | 1970 | 1971 | 1972 | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1950 | 1950 | 1951 | 1952 | 1953 | 1954 | 1955 | 1956 | 1957 | 1958 | 1959 | 1960 | 1961 | 1962 | 1963 | 1964 | 1965 | 1966 | 1967 | 1968 | 1969 | 1970 | 1971 | 1972 | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 |

Summary of the Invention

In preferred embodiments, the polypeptide is a recombinant or a substantially pure
15 preparation of an Helios polypeptide.

In preferred embodiments, the polypeptide is a vertebrate, e.g., a mammalian, e.g., a human polypeptide.

In preferred embodiments, the Helios polypeptide includes additional Helios coding sequences 5' to that of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

20 In preferred embodiments: the polypeptide has at least one biological activity, e.g., it
reacts with an antibody, or antibody fragment, specific for an Helios polypeptide; the
polypeptide includes an amino acid sequence at least 60%, 74%, 80%, 90%, 95%, 98%, or
99% homologous to an amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID
NO:6; the polypeptide includes an amino acid sequence essentially the same as an amino
25 acid sequence in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6; the polypeptide is at least
5, 10, 20, 50, 100, 150, 200, or 250 amino acids in length; the polypeptide includes at least
5, preferably at least 10, more preferably at least 20, most preferably at least 50, 100, 150,
200, or 250 contiguous amino acids from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6;
the polypeptide is preferably at least 10, but no more than 100, amino acids in length; the
30 Helios polypeptide is either, an agonist or an antagonist, of a biological activity of a
naturally occurring Helios polypeptide.

In preferred embodiments: the Helios polypeptide is encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6, or by a nucleic acid having at least 60%, 70%, 80%, 90%, 95%, 98%, or 99% homology with the nucleic acid of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6. For example, the Helios polypeptide can be
5 encoded by a nucleic acid sequence which differs from a nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6 due to degeneracy in the genetic code.

In a preferred embodiment, the Helios polypeptide encodes amino acid residues 1-526 of SEQ ID NO:2, residues 1-500 of SEQ ID NO:4 or residues 1-526 of SEQ ID NO:6 or a functionally equivalent residue in the Helios sequence of another vertebrate or
10 mammal, e.g., a monkey.

In a preferred embodiment the Helios polypeptide is an agonist of a naturally-occurring mutant or wild type Helios polypeptide (e.g., a polypeptide having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6). In another preferred embodiment, the polypeptide is an antagonist which, for example, inhibits an undesired
15 activity of a naturally-occurring Helios polypeptide (e.g., a mutant polypeptide).

In a preferred embodiment, the Helios polypeptide differs in amino acid sequence at 1, 2, 3, 5, 10 or more residues, but preferably less than 15, from a sequence in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6. The differences, however, are such that the Helios polypeptide exhibits at least one biological activity of an Helios polypeptide, e.g., the Helios
20 polypeptide retains a biological activity of a naturally occurring Helios polypeptide. In other preferred embodiments, the Helios polypeptide differs at up to 1, 2, 3, 5, 10 amino acid residues from the sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

In preferred embodiments the Helios polypeptide includes an Helios polypeptide sequence, as described herein, as well as other N-terminal and/or C-terminal amino acid
25 sequences.

In preferred embodiments, the polypeptide includes all or a fragment of an amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, fused, in reading frame, to additional amino acid residues, preferably to residues encoded by genomic DNA 5' to the genomic DNA which encodes a sequence from SEQ ID NO:2, SEQ ID NO:4, or
30 SEQ ID NO:6.

In another aspect, the invention features a fragment of an Helios polypeptide. In one embodiment, the fragment is a terminal fragment, e.g., an N- or C- terminal deletion, e.g., a zinc finger, or an internal deletion, e.g., a zinc finger or a transcriptional activation domain. In another embodiment, the fragment includes one or more of: a N-terminal zinc finger, e.g.,
5 N-zinc finger 1 (ZF1), N-zinc finger 2 (ZF2), N-zinc finger 3 (ZF3), N-zinc finger 4 (ZF4), a transcriptional activation domain, or a C-terminal zinc finger, e.g., C-zinc finger 1 (ZF5), C-zinc finger 2 (ZF6). In another embodiment, the Helios polypeptide includes a deletion of one or more of the following: a N-terminal zinc finger, e.g., N-zinc finger 1 (ZF1), N-zinc finger 2 (ZF2), N-zinc finger 3 (ZF3), N-zinc finger 4 (ZF4), a transcriptional
10 activation domain, or a C-terminal zinc finger, .e.g., a C-zinc finger 1 (ZF5) or a C-zinc finger 2 (ZF6). In another embodiment, the fragment is at least 20, 40, 60, or 80 amino acids in length.

In yet other preferred embodiments, the Helios polypeptide is a recombinant fusion protein having a first Helios polypeptide portion and a second polypeptide portion having an
15 amino acid sequence unrelated to an Helios polypeptide. The second polypeptide portion can be, e.g., any of glutathione-S-transferase, a DNA binding domain, or a polymerase activating domain. In preferred embodiment the fusion protein can be used in a two-hybrid assay.

In a preferred embodiment, the Helios polypeptide is a fragment or analog of a
20 naturally occurring Helios polypeptide which inhibits reactivity with antibodies, or F(ab')₂ fragments, specific for a naturally occurring Helios polypeptide.

In a preferred embodiment, the Helios polypeptide includes a sequence which is not present in the mature protein.

Polypeptides of the invention include those which arise as a result of the existence of
25 multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events.

In preferred embodiments, the Helios polypeptide has one or more of the following properties:

- (a) it can form a dimer with an Helios, Aiolos, or Ikaros polypeptide;
- 30 (b) it is expressed in hematopoietic stem cells;
- (c) it has a molecular weight of approximately 64 kDa or 68 KDa;

- (d) it has at least one zinc finger domain; or
- (e) it is a transcriptional activator of a lymphoid gene.

The invention includes an immunogen which includes an active or inactive Helios polypeptide, or an analog or a fragment thereof, in an immunogenic preparation, the immunogen being capable of eliciting an immune response specific for the Helios polypeptide, e.g., a humoral response, an antibody response, or a cellular response. In preferred embodiments, the immunogen comprising an antigenic determinant, e.g., a unique determinant, from a protein represented by SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

The invention also includes an antibody preparation, preferably a monoclonal antibody preparation, specifically reactive with an epitope of the Helios immunogen or generally of an Helios polypeptide.

In another aspect, the invention provides a substantially pure nucleic acid having, or comprising, a nucleotide sequence which encodes a polypeptide, the amino acid sequence of which includes, or is, the sequence of an Helios polypeptide, or analog or fragment thereof.

In preferred embodiments, the nucleic acid encodes a vertebrate, e.g., a mammalian, e.g., a human polypeptide.

In preferred embodiments, the nucleic acid encodes an Helios polypeptide which includes additional Helios coding sequences 5' to that SEQ ID NO:2, 4, or 6.

In preferred embodiments, the nucleic acid encodes a polypeptide having one or more of the following characteristics: at least one biological activity of an Helios, e.g., a polypeptide specifically reactive with an antibody, or antibody fragment, directed against an Helios polypeptide; an amino acid sequence at least 60%, 74%, 80%, 90%, 95%, 98%, or 99% homologous to an amino acid sequence from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6; an amino acid sequence essentially the same as an amino acid sequence in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, the polypeptide is at least 5, 10, 20, 50, 100, 150, 200, or 250 amino acids in length; at least 5, preferably at least 10, more preferably at least 20, most preferably at least 50, 100, 150, 200, or 250 contiguous amino acids from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6; an amino acid sequence which is preferably at least 10, but no more than 100, amino acids in length; the ability to act as an agonist or an antagonist of a biological activity of a naturally occurring Helios polypeptide.

In preferred embodiments: the nucleic acid is or includes the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6; the nucleic acid is at least 60%, 70%, 74%, 80%, 90%, 95%, 98%, or 99% homologous with a nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6; the nucleic acid includes a fragment of SEQ ID NO:1,
5 SEQ ID NO:3, or SEQ ID NO:6 which is at least 25, 50, 100, 200, 300, 400, 500, or 1,000 bases in length; the nucleic acid differs from the nucleotide sequence of SEQ ID NO:1 due to degeneracy in the genetic code.

In a preferred embodiment, the Helios encoding nucleic acid sequence encodes amino acid residues 1-526 of SEQ ID NO:2, residues 1-500 of SEQ ID NO:4, residues 1-
10 526 of SEQ ID NO:6 or a functionally equivalent residue in the Helios sequence of another vertebrate or mammal, e.g., a monkey.

In a preferred embodiment the polypeptide encoded by the nucleic acid is an agonist which, for example, is capable of enhancing an activity of a naturally-occurring mutant or wild type Helios polypeptide. In another preferred embodiment, the encoded polypeptide is
15 an antagonist which, for example, inhibits an undesired activity of a naturally-occurring Helios polypeptide (e.g., a polypeptide having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6).

In a preferred embodiment, the encoded Helios polypeptide differs in amino acid sequence at 1, 2, 3, 5, 10 or more residues, but preferably less than 15, from a sequence in
20 SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6. The differences, however, are such that the encoded Helios polypeptide exhibits at least one biological activity of a naturally occurring Helios polypeptide (e.g., the Helios polypeptide of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6).

In preferred embodiments, the nucleic acid encodes an Helios polypeptide which
25 includes an Helios polypeptide sequence, as described herein, as well as other N-terminal and/or C-terminal amino acid sequences.

In preferred embodiments, the nucleic acid encodes a polypeptide which includes all or a portion of an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, fused, in reading frame, to additional amino acid residues, preferably to residues
30 encoded by genomic DNA 5' to the genomic DNA which encodes a sequence from SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

In preferred embodiments, the encoded polypeptide is a recombinant fusion protein having a first Helios polypeptide portion and a second polypeptide portion having an amino acid sequence unrelated to an Helios polypeptide. The second polypeptide portion can be, e.g., any of glutathione-S-transferase; a DNA binding domain; or a polymerase activating domain. In preferred embodiments the fusion protein can be used in a two-hybrid assay.

In preferred embodiments, the encoded polypeptide is a fragment or analog of a naturally occurring Helios polypeptide which inhibits reactivity with antibodies, or F(ab')₂ fragments, specific for a naturally occurring Helios polypeptide.

In preferred embodiments, the nucleic acid will include a transcriptional regulatory sequence, e.g. at least one of a transcriptional promoter or transcriptional enhancer sequence, operably linked to the Helios gene sequence, e.g., to render the Helios gene sequence suitable for use as an expression vector.

In yet another preferred embodiment, the nucleic acid of the invention hybridizes under stringent conditions to a nucleic acid probe corresponding to at least 12 consecutive nucleotides from SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6, or more preferably to at least 20 consecutive nucleotides from SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6, or more preferably to at least 40 consecutive nucleotides from SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6.

In a preferred embodiment, the nucleic acid encodes an Helios polypeptide which includes a sequence which is not present in the mature protein.

In preferred embodiments, the nucleic acid encodes an Helios polypeptide which has one or more of the following properties:

- (a) it can form a dimer with an Helios, Aiolos, or Ikaros polypeptide;
- (b) it is expressed in hematopoietic stem cells;
- (c) it has a molecular weight of approximately 64 kDa or 68 KDa;
- (d) it has at least one zinc finger domain; or
- (e) it is a transcriptional activator of a lymphoid gene.

In another aspect, the invention includes: a vector including a nucleic acid which encodes an Helios polypeptide; a host cell transfected with the vector; and a method of

producing a recombinant Helios polypeptide, including culturing the cell, e.g., in a cell culture medium, and isolating the Helios polypeptide, e.g., an Helios polypeptide from the cell or from the cell culture medium.

5 In another aspect, the invention features, a purified recombinant nucleic acid having at least 50%, 60%, 70%, 74%, 80%, 90%, 95%, 98%, or 99% homology with a nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6.

10 The invention also provides a probe or primer which includes or comprises a substantially purified oligonucleotide. The oligonucleotide includes a region of nucleotide sequence which hybridizes under stringent conditions to at least 10 consecutive nucleotides of sense or antisense sequence from SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6, or naturally occurring mutants thereof. In preferred embodiments, the probe or primer further includes a label group attached thereto. The label group can be, e.g., a radioisotope, a fluorescent compound, an enzyme, and/or an enzyme co-factor. Preferably the oligonucleotide is at least 10 and less than 20, 30, 50, 100, or 150 nucleotides in length.

15 The invention involves nucleic acids, e.g., RNA or DNA, encoding a polypeptide of the invention. This includes double stranded nucleic acids as well as coding and antisense single strands.

The invention includes vertebrate, e.g., mammalian, e.g., rodent, e.g., mouse or rat, or human Helios polypeptides.

20 In another aspect, the invention features a method of evaluating a compound for the ability to interact with, e.g., bind, or modulate, e.g., inhibit or promote, the activity of an Helios polypeptide, e.g., an Helios monomer, or an Helios-Helios dimer, an Helios-Aiolos dimer, or an Helios-Ikaros dimer. The method includes contacting the compound with the Helios polypeptide, and evaluating the ability of the compound to interact with or form a complex with the Helios polypeptide. This method can be performed *in vitro*, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with the Helios polypeptide. It can also be used to find natural or synthetic inhibitors of mutant or wild type Helios polypeptide. The compound can be a peptide or a non peptide molecule, e.g., a small molecule preferably 500 to 5,000 molecular weight, more preferably 500 to 1,000 molecular

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weight, having an aromatic scaffold, e.g., a bis-amide phenol, decorated with various functional groups.

In brief, a two hybrid assay system (see e.g., Bartel et al. (1993) *Cellular Interaction in Development: A practical Approach*, D.A. Hartley, ed., Oxford University Press, Oxford, pp. 153-179) allows for detection of protein-protein interactions in yeast cells. The known protein, e.g., an Helios polypeptide, is often referred to as the "bait" protein. The proteins tested for binding to the bait protein are often referred to as "fish" proteins. The "bait" protein, e.g., an Helios polypeptide, is fused to the GAL4 DNA binding domain. Potential "fish" proteins are fused to the GAL4 activating domain. If the "bait" protein and a "fish" protein interact, the two GAL4 domains are brought into close proximity, thus rendering the host yeast cell capable of surviving a specific growth selection.

In another aspect, the invention features a method of identifying active fragments or analogs of an Helios polypeptide. The method includes first identifying a compound, e.g., an Ikaros peptide, which interacts with an Helios polypeptide and determining the ability of the compound to bind the candidate fragment or analog. The two hybrid assay described above can be used to obtain fragment-binding compounds. These compounds can then be used as "bait" to fish for and identify fragments of the Helios polypeptide which interact, bind, or form a complex with these compounds.

In another aspect, the invention features a method of making an Helios polypeptide, having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring Helios polypeptide. The method includes altering the sequence of an Helios polypeptide (e.g., SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6) by, for example, substitution or deletion of one or more residues of a non-conserved region, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of an Helios polypeptide, e.g., an Helios polypeptide having at least one biological activity of a naturally occurring Helios polypeptide. The method includes altering the sequence, e.g., by substitution or deletion of one or more residues, preferably which are non-conserved residues, of an Helios polypeptide, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features, a method of evaluating a compound for the ability to bind a nucleic acid encoding an Helios gene regulatory sequence. The method includes: contacting the compound with the nucleic acid; and evaluating ability of the compound to form a complex with the nucleic acid. In preferred embodiments the Helios gene regulatory sequence is functionally linked to a heterologous gene, e.g., a reporter gene.

In another aspect, the invention features a human cell, e.g., a hematopoietic stem cell or a lymphocyte e.g., a T or a B cell, transformed with a nucleic acid which encodes an Helios polypeptide.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder, e.g., an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering a therapeutically-effective amount of an Helios polypeptide to the animal. The Helios polypeptide can be monomeric or an Helios-Helios, an Helios-Aiolos dimer, or Helios-Ikaros dimer.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse. The method includes administering to the animal a cell selected, e.g., selected in vitro, for the expression of a product of the Helios gene, e.g., hematopoietic stem cells, e.g., cells transformed with Helios-peptide-encoding DNA, e.g., hematopoietic stem cells transformed with Helios-peptide-encoding DNA.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse. The method

includes administering to the animal a nucleic acid encoding an Helios peptide and expressing the nucleic acid.

In another aspect, the invention features a method of evaluating the effect of a treatment, e.g., a treatment designed to promote or inhibit hematopoiesis, including carrying
5 out the treatment and evaluating the effect of the treatment on the expression of the Helios gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, or a cell, e.g., a cultured
10 stem cell.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Helios gene or a disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells. The method includes examining the
15 subject for the expression of the Helios gene, non-wild type expression or mis-expression being indicative of risk.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Helios gene or a disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a
20 disorder characterized by a shortage of T or B cells. The method includes providing a nucleic acid sample from the subject and determining if the structure of an Helios gene allele of the subject differs from wild type.

In preferred embodiments: the determination includes determining if an Helios gene allele of the subject has a gross chromosomal rearrangement; the determination includes
25 sequencing the subject's Helios gene.

In another aspect, the invention features, a method of evaluating an animal or cell model for a proliferative disorder, e.g., a leukemic disorder, Hodgkin's lymphoma, a cutaneous cell lymphoma, e.g., a cutaneous T cell lymphoma, or an immune disorder, e.g., a T cell related disorder, e.g., a disorder characterized by a shortage of T or B cells. The
30 method includes determining if the Helios gene in the animal or cell model is expressed at a predetermined level or if the Helios gene is mis-expressed. In preferred embodiments: the

predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal; or the pattern of isoform expression is altered from wildtype.

In another aspect, the invention features, a transgenic animal, e.g., a mammal, e.g., a mouse or a nonhuman primate having an Helios transgene.

In preferred embodiments the animal is a transgenic mouse having a mutated Helios transgene, the mutation occurring in, or altering, e.g., a domain of the Helios gene described herein.

In preferred embodiments the transgenic animal, e.g., a transgenic mouse, is homozygous for null mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus.

In preferred embodiments the transgenic animal, e.g., a transgenic mouse, is homozygous for null mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus and includes a mutation at Ikaros or Aiolos, e.g., a dominant negative mutation at Ikaros or Aiolos. Preferably the Ikaros mutation is heterozygous.

In other preferred embodiments the transgenic animal or cell: is heterozygous for an Helios transgene; homozygous for an Helios transgene; includes a first Helios transgene and a second Helios transgene; includes an Helios transgene and a second transgene which is other than an Helios transgene, e.g., an Ikaros or Aiolos transgene.

In another aspect, the invention features a method for evaluating the effect of a treatment on a transgenic cell or animal having an Helios transgene, e.g., the effect of the treatment on the development of the immune system. The method includes administering the treatment to a cell or animal having an Helios transgene, and evaluating the effect of the treatment on the cell or animal. The effect can be, e.g., the effect of the treatment on: Helios or Ikaros expression or misexpression; the immune system or a component thereof; or the cell cycle. Immune system effects include e.g., T cell activation, T cell development, the ability to mount an immune response, the ability to give rise to a component of the

immune system, B cell development, NK cell development, or the ratios $CD4^+/CD8^+$, $CD4^+/CD8^-$ and $CD4^-/CD8^+$.

In preferred embodiments the treatment can include: the administration of a drug, chemical, or other substance; the administration of ionizing radiation; the administration of
5 an antibody, e.g., an antibody directed against a molecule or cell of the immune system; administration of a substance or other treatment which suppresses the immune system; or administration of a substance or other treatment which activates or boosts the function of the immune system; introduction of a nucleic acid, e.g., a nucleic acid which encodes or expresses a gene product, e.g., a component of the immune system; the introduction of a
10 protein, e.g., a protein which is a component of the immune system.

In another aspect, the invention features a method for evaluating the effect of a treatment on an immune system component. The method includes: (1) supplying a transgenic cell or animal having an Helios transgene; (2) supplying the immune system component; (3) administering the treatment; and (4) evaluating the effect of the treatment
15 on the immune system component.

In yet another aspect, the invention features a method for evaluating the interaction of a first immune system component with a second immune system component. The method includes: (1) supplying a transgenic cell or animal, e.g., a mammal, having an Helios transgene; (2) introducing the first and second immune system component into the
20 transgenic cell or mammal; and (3) evaluating an interaction between the first and second immune system components.

In another aspect, the invention features a method for evaluating the effect of a treatment on an immune system disorder, e.g., a neoplastic disorder, a leukemia or a lymphoma, a T cell related lymphoma, including: administering the treatment to a cell or
25 animal having an Helios transgene, and evaluating the effect of the treatment on the cell or animal. The effect can be, e.g., the effect of the treatment on: Helios or Ikaros expression or misexpression; the immune system or a component thereof; or the cell cycle. Immune system effects include e.g., T cell activation, T cell development, the ability to mount an immune response, the ability to give rise to a component of the immune system, B cell

development, NK cell development, or the ratios $CD4^+/CD8^+$, $CD4^+/CD8^-$ and $CD4^-/CD8^+$.

The inventors have also discovered that Ikaros and Helios can form dimers (heterodimers) with other polypeptides. E.g., an Ikaros polypeptide can form dimers not only with Ikaros polypeptides, but with other polypeptides which bind to its C terminal region, e.g, other polypeptides having Zinc-finger regions, e.g., Helios polypeptides. Similarly, an Helios polypeptide can form dimers not only with Helios polypeptides, but with other polypeptides which bind to its C terminal region, e.g, other polypeptides having Zinc-finger regions, e.g., Ikaros polypeptides.

The invention also includes Ikaros-Helios or Aiolos/Helios dimers. The Ikaros member of the dimer can be any Ikaros polypeptide, e.g., any naturally occurring Ikaros or any Ikaros referred to in U.S.S.N.08/238,212, filed May 2, 1994, hereby incorporated by reference. The Aiolos member of the dimer can be any Aiolos polypeptide, e.g., any naturally occurring Aiolos or any Aiolos referred to in U.S.S.N.60/005,529 filed October 18, 1995, hereby incorporated by reference.

The invention also includes: a cell, e.g., a cultured cell or a stem cell, containing purified Ikaros- or Aiolos-protein-encoding-DNA and purified Helios-protein-encoding -DNA; a cell capable of expressing an Ikaros and an Helios protein; a cell capable of giving rise to a transgenic animal or to a homogeneous population of hemopoietic cells, e.g., lymphoid cells, e.g., T cells; an essentially homogeneous population of cells, each of which includes purified Ikaros- or Aiolos-protein-encoding-DNA and purified Helios-protein-encoding -DNA ; and a method for manufacture of a dimer of the invention including culturing a cell which includes a DNA, preferably a purified DNA, of the invention in a medium to express the peptides .

The invention also includes: a preparation of cells, e.g., cultured cells or a stem cells, including a cell a containing purified Ikaros- or Aiolos-protein-encoding-DNA and a cell encoding purified Helios-protein-encoding -DNA.

The invention also includes substantially pure preparation of an antibody, preferably a monoclonal antibody directed against an Ikaros-Helios dimer or an Aiolos-Helios dimer (which preferably does not bind to an Ikaros-Ikaros, Aiolos-Aiolos or Helios-Helios dimer); a therapeutic composition including an Ikaros-Helios dimer or an Aiolos-Helios dimer and a

pharmaceutically acceptable carrier; a therapeutic composition which includes a purified DNA of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering a therapeutically-effective amount of an Ikaros-Helios or an Aiolos-Helios dimer to the animal.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse including administering to the animal cells selected, e.g., selected in vitro, for the expression of a product of the Ikaros gene and of the Helios gene, e.g., hematopoietic stem cells, e.g., cells transformed with Ikaros- or Aiolos-peptide-encoding DNA and or Helios-peptide-encoding DNA, e.g., hematopoietic stem cells transformed with Ikaros or Aiolos and or Helios-peptide-encoding DNA. The Ikaros Aiolos and Helios DNA can be present in the same or in different cells.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering to the animal a nucleic acid encoding an Ikaros peptide and a nucleic acid encoding an Helios peptide and expressing the nucleic acids.

In another aspect, the invention features a method of evaluating the effect of a treatment, e.g., a treatment designed to promote or inhibit hematopoiesis, including carrying out the treatment and evaluating the effect of the treatment on the expression of the Ikaros and the Helios gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, or a cell, e.g., a cultured stem cell.

5 In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a leukemic disorder or other disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including
10 examining the subject for the expression of the Ikaros-Helios or Aiolos-Helios dimers, non-wild type expression or mis-expression being indicative of risk.

In another aspect, the invention features, a method of evaluating an animal or cell model for an immune disorder, e.g., a T cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including determining if Ikaros-Helios or Aiolos-Helios dimers in the animal or cell model are expressed at a predetermined level. In preferred
15 embodiments: the predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal; or the pattern of isoform expression is altered from wildtype.

In another aspect, the invention features a transgenic rodent, e.g., a mouse, having a transgene which includes an Ikaros or Aiolos gene or Ikaros or Aiolos protein encoding
20 DNA and an Helios gene or Helios protein encoding DNA. In preferred embodiments: the Ikaros, Aiolos and or Helios gene or DNA includes a deletion, e.g. a deletion of all or part of one or more exons.

In another aspect, the invention features, a method of culturing an Helios-misexpressing cell having at least one mutant allele at the Helios locus. The cell can be,
25 e.g., a hematopoietic cell, e.g., a T lymphocyte. The method includes: introducing the cell into a mammal, wherein, preferably, the mammal is other than the one from which the cell has been isolated originally; and culturing the cell.

In a preferred embodiment, the method further includes: allowing the cell to proliferate in the mammal.

30 In preferred embodiments: the mammal is a non-human mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: allowing the Helios-misexpressing cell to divide and give rise to a proliferation-deregulated cell, e.g., a transformed lymphocyte; providing a plurality of the proliferation-deregulated cells e.g., lymphocytes or transformed lymphocytes from the mammal.

5 In preferred embodiments: the mammal, the cell or both, are heterozygous at the Helios locus; the mammal, the cell or both, carry a mutation at the Helios gene, e.g., a point mutation in or a deletion for all or part of the Helios gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Helios protein or
10 for one or more of the two C terminal zinc finger regions which mediate dimerization of the Helios protein; the mammal is heterozygous or homozygous for an Helios transgene; the mammal, the cell or both, carry a mutation in the control region of the Helios gene.

In preferred embodiments: the mammal, the cell or both, carry a mutation at the Helios gene, e.g., a point mutation or a deletion, which, inactivates one or both of
15 transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains.

In preferred embodiments the cell, e.g., a cell, is homozygous for null mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus.

In preferred embodiments the cell, e.g., a mouse cell, is homozygous for null
20 mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus and includes a mutation at Ikaros, e.g., a dominant negative mutation at Ikaros. Preferably the Ikaros mutation is heterozygous.

In preferred embodiments: the Helios-misexpressing cell is a homozygous mutant Helios cell e.g., a lymphocyte; the Helios-misexpressing cell is a B lymphocyte; the Helios-
25 misexpressing cell is heterozygous or homozygous for an Helios transgene.

In preferred embodiments, the Helios-misexpressing cell is a lymphocyte and is: a cell which secretes one or more anti-inflammatory cytokines; a cell which is antigen or idiotype specific.

In preferred embodiments: the mammal is immunized with an antigen; the cell is
30 exogenously supplied and one or both of the mammal or the mammal which donates the cell

are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen; an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In a preferred embodiment: the Helios-misexpressing cell, e.g., a lymphocyte, is supplied exogenously to the mammal, e.g., to a homozygous wild-type Helios mammal or a mammal carrying a mutation at the Helios gene, e.g., a point mutation or a deletion for all or part of the Helios gene. If exogenously supplied, the cell can be a human or a nonhuman, e.g., a swine, nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, lymphocyte.

Helios wild type cells can be cultured in Helios misexpressing mammals.

In another aspect, the invention features, a method of modulating the activity of, or promoting the interaction of an Helios misexpressing cell with, a target tissue or cell. The method includes: supplying the target; and exposing the target to a Helios misexpressing cell, e.g., a hematopoietic cell, e.g., a T lymphocyte, preferably having at least one mutant allele at the Helios locus, preferably provided that: the target is not Helios-misexpressing; the target and the cell differ in genotype at a locus other than the Helios locus; the target and the cell are from different animals; the target and the cell are from different species; the target activity is modulated in a recipient mammal and either the target or the cell is from a donor mammal other than the recipient mammal; or the target is exposed to the cell in an *in vitro* system.

In a preferred embodiment: the donor of the Helios-misexpressing cell is heterozygous or homozygous for an Helios transgene; the donor of the Helios-misexpressing cell is heterozygous at the Helios locus; the donor of the Helios-misexpressing cell carries a point mutation in or a deletion for all or part of the Helios gene, e.g., mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediate Helios binding to DNA or in one or both of the C-terminal zinc finger regions which mediates Helios dimerization; the donor of the Helios-misexpressing cell is human or a non-human mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse. In preferred embodiments, e.g., in the case of the human donor, the manipulation that gives rise to Helios deregulation, e.g., an Helios lesion, can be made *in vitro*.

In preferred embodiments: the mammal which provides the Helios misexpressing cell carries a mutation at the Helios gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains.

In another preferred embodiment: the cell is heterozygous or homozygous for an Helios transgene; the cell is a heterozygous Helios cell; the cell is a homozygous mutant Helios cell; the lymphocyte is a T lymphocyte.

In preferred embodiments, the cell is a lymphocyte and is: a T cell; a cell which secretes one or more anti-inflammatory cytokines; a T cell which is antigen or idiotype specific.

In a preferred embodiment: the method is performed in an *in vitro* system; the method is performed *in vivo*, e.g., in a mammal, e.g., a rodent, e.g., a mouse or a rat, or a primate, e.g., a non-human primate or a human. If the method is performed *in vitro*, the donor of the target cell or tissue and the lymphocyte can be same or different. If the method is performed *in vivo*, there is a recipient animal and one or more donors.

In preferred embodiments: the method is performed *in vivo* and one or more of the recipient, the donor of the target cell or tissue, the donor of the cell, is immunized with an antigen; the method is performed *in vitro* and one or more of the donor of the target cell or tissue, the donor of the cell is immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In a preferred embodiment: the target is selected from a group consisting of T or B lymphocytes, macrophages, inflammatory leukocytes, e.g., neutrophils or eosinophils, mononuclear phagocytes, NK cells or T lymphocytes; the target is an antigen presenting cell, e.g., a professional antigen presenting cell or a nonprofessional antigen presenting cell; the target is spleen tissue, bone marrow tissue, lymph node tissue or thymic tissue, or the target is a syngeneic, allogeneic, or xenogeneic tissue.

In another preferred embodiment, the target is from a mammal, e.g., a human; the mammal is a non-human mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In preferred embodiments, the activity of the target which is modulated is: the production of a cytokine; the proliferation or activation of a cell of the immune system; the production of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T lymphocyte; the effect of target on resistance to infection; the effect of target on life span; the effect of target on body weight; the effect of target on the presence, function, or morphology of tissues or organs of the immune system; the effect of target on the ability of a component of the immune system to respond to a stimulus (e.g., a diffusable substance, e.g., cytokines, other cells of the immune system, or antigens); the effect of target on the ability to exhibit immunological tolerance to an alloantigen or a xenoantigen.

In preferred embodiments the interaction is the binding of an antibody produced by the Helios misexpressing cell with the target.

In preferred embodiments: the target and the cell differ in genotype at a locus other than the Helios locus; the target and the cell are from different animals; the target is not Helios-misexpressing.

In another aspect, the invention features, a method of reconstituting an immune system. The method includes: supplying a recipient mammal, and introducing, preferably exogenously, into the recipient mammal, an immune system component from a donor mammal, which is Helios misexpressing, e.g., which carries at least one mutant allele at the Helios locus. The recipient mammal, can be, e.g., a human or a nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse. The donor mammal can be, e.g., a human or a nonhuman mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse. If the donor mammal is human, the manipulation that gives rise to Helios misexpression e.g., an the introduction of an Helios lesion, can be made *in vitro*. The donor mammal and the recipient mammal can be different individuals or the same individual.

In preferred embodiments, the component is or includes an Helios misexpressing cell, e.g., a hematopoietic cell, e.g., a pluripotent stem cell, or a descendent of a stem cell, e.g., a lymphocyte.

In preferred embodiments, the component is from a donor mammal, e.g., a human or a nonhuman mammal, e.g., a swine, a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: prior to introduction of a component into the subject, treating the lymphocyte to inhibit proliferation, e.g., by
5 irradiating the component.

In a preferred embodiment, the donor mammal carries a mutation at the Helios gene, e.g., a deletion of all or part of the Helios gene.

In another preferred embodiment: the immune system component is any of a T cell, a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem
10 cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue.

In a preferred embodiment: the immune system component is from the same species as the recipient mammal; the immune system component is from species different from the species of the recipient mammal.

In preferred embodiments: the recipient mammal is a wild-type animal; an animal
15 model for a human disease, e.g., a NOD mouse; the animal is immunocompromised by irradiation, chemotherapy, or genetic defect, e.g., the animal is a SCID mouse or a nude mouse; the recipient is deficient in an immune function, e.g., the recipient has been thymectomized, depleted of an immune system component, e.g., of cells or antibodies; the
20 recipient has been administered chemotherapy or irradiation.

In preferred embodiments: the immune system component is heterozygous at the Helios locus; the immune system component is carries a mutation at the Helios gene, e.g., a point mutation in or a deletion for all or part of the Helios gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the
25 four N-terminal zinc finger regions which mediates DNA binding of the Helios protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Helios protein; the immune system component is heterozygous or homozygous for an Helios transgene; the immune system component carries a mutation in the control region of the Helios gene.

In preferred embodiments: the immune system component carries a mutation at the
30 Helios gene, e.g., a point mutation or a deletion, which, inactivates one or both of

transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains.

In preferred embodiments: the method is performed *in vivo*, and the recipient mammal or the donor mammal or both are immunized with an antigen. The antigen can be:
5 an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In a preferred embodiment, the method further includes: determining a value for a parameter related to immune system function. The parameter related to the immune system function can be any of: the production of a cytokine; the proliferation or activation of a cell
10 of the immune system; the production of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T lymphocyte; resistance to infection; life span; body weight; the presence, function, or morphology of tissues or organs of the immune system; the ability of a component of the immune system to respond to a stimulus (e.g., a diffusable substance, e.g., cytokines, other cells of the immune system, or antigens); the ability to present an
15 antigen; the ability to exhibit immunological tolerance to an alloantigen or a xenoantigen.

In another aspect, the invention features, a method of evaluating the interaction of an Helios misexpressing cell, e.g., a hematopoietic cell, a T lymphocyte, with an immune system component. The method includes: supplying an animal, e.g., a swine, a nonhuman
20 primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse; introducing the cell and the immune component into the animal; and evaluating the interaction between the Helios misexpressing cell and the immune system component.

In a preferred embodiment, the method further includes: prior to introduction of a cell into the subject, treating the lymphocyte to inhibit proliferation, e.g., by irradiating the
25 cell.

In a preferred embodiment: the immune system component is any of a T cell, a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue; the immune system component is from the same
30 species as the animal; the immune system component is from species different from the species of the animal; the immune system component is from the same species as the

lymphocyte; the immune system component is from species different from the species from which the lymphocyte is obtained.

In another preferred embodiment: the cell is from the same species as the animal; the cell is from a species which is different from the species of the animal.

5 In another preferred embodiment: the recipient mammal is a wild-type animal; an animal model for a human disease, e.g., a NOD mouse; the animal is immunocompromised by irradiation, chemotherapy, or genetic defect, e.g., the animal is a SCID mouse or a nude mouse; the recipient is deficient in an immune function, e.g., the recipient has been thymectomized, depleted of an immune system component, e.g., of cells or antibodies; the
10 recipient has been administered chemotherapy or irradiation.

In a preferred embodiment: the cell is heterozygous or homozygous for an Helios transgene.

In preferred embodiments evaluating can include evaluating any of: the production of a cytokine; the proliferation or activation of a cell of the immune system; the production
15 of an antibody; the lysis of an antigen presenting cell or the activation of a cytolytic T lymphocyte; resistance to infection; life span; body weight; the presence, function, or morphology of tissues or organs of the immune system; the ability of a component of the immune system to respond to a stimulus (e.g., a diffusable substance, e.g., cytokines, other cells of the immune system, or antigens); the ability to present an antigen; the ability to
20 exhibit immunological tolerance to an alloantigen or a xenoantigen.

In preferred embodiments: the method is performed *in vivo*, and one or more of the animal, the donor of the Helios misexpressing cell, the donor of the immune system component, is immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic
25 lymphocyte.

In another aspect, the invention features, a mammal, e.g., a nonhuman mammal, e.g., e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, having an exogenously introduced immune system component, the component being from a
30 human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, or cell culture which is Helios misexpressing or which

carries at least one mutant allele at the Helios locus. In preferred embodiments, e.g., if the immune system component is from a wild-type animal, e.g., a human, the manipulation that gives rise to Helios deregulation, e.g., an Helios lesion, can be made *in vitro*.

In preferred embodiments, the component is from a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, which is Helios misexpressing.

In preferred embodiments: the component is from a mammal which is Helios misexpressing; the component is from a mammal which is heterozygous at the Helios locus; the component is from a mammal which carries a mutation at the Helios gene, e.g., a point mutation in or a deletion for all or part of the Helios gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediates DNA binding of the Helios protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Helios protein; the component is from a mammal which is heterozygous or homozygous for an Helios transgene; the component is from a mammal which carries a mutation in the control region of the Helios gene.

In preferred embodiments: the component is from a mammal which carries a mutation at the Helios gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains.

In preferred embodiments, the immune system component is: a helper T cell; cytolytic T cell; a suppressor T cell; a T cell which secretes one or more anti-inflammatory cytokines, e.g., IL-4, IL-10, or IL-13; a T cell which is antigen or idiotypic specific; a suppressor T cell which is anti-idiotypic for an auto antibody or for an antibody which recognizes an allograft or xenograft tissue; the lymphocyte is an antigen-nonspecific T cell.

In another preferred embodiment: the immune system component is any of a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue; the immune system component is from the same species as the animal; the immune system component is from species different from the species of the animal.

In preferred embodiments: the mammal or the donor animal which produces the immune system component or both are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

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In another aspect, the invention features, a reaction mixture, preferably an *in vitro* reaction mixture, including an immune system component, the component including cells which misexpress Helios or being from an animal or cell culture which misexpresses Helios or which carries at least one mutant allele at the Helios locus, and a target tissue or cell, wherein preferably, the immune system component and the target differ in genotype at a locus other than the Helios or Ikaros locus; the component and the target are from different species, or the component and the target are from different animals.

10

In preferred embodiments, the component is from an animal or cell culture which misexpresses Helios.

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In preferred embodiments: the immune system component is a lymphocyte heterozygous or homozygous for an Helios transgene, e.g., a transgene having a point mutation or a deletion, which, inactivates one or both of transcriptional activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains; the immune system component is a lymphocyte heterozygous or homozygous for a C terminal deletion.

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In preferred embodiments, the immune system component is: a B cell.

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In another preferred embodiment: the immune system component is any of a T cell progenitor, a totipotent hematopoietic stem cell, a pluripotent hematopoietic stem cell, a B cell, a B cell progenitor, a natural killer cell, a natural killer cell progenitor, bone marrow tissue, spleen tissue, or thymic tissue; the immune system component is from the same species as the target cell; the immune system component is from species different from the species of the target cell.

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In a preferred embodiment: the target is selected from a group consisting of T or B lymphocytes, macrophages, inflammatory leukocytes, e.g., neutrophils or eosinophils, mononuclear phagocytes, NK cells or T lymphocytes; the target is an antigen presenting cell, e.g., a professional antigen presenting cell or a nonprofessional antigen presenting cell;

the target is spleen tissue, lymph node tissue, bone marrow tissue or thymic tissue, or is syngeneic, allogeneic, xenogeneic, or congenic tissue.

In preferred embodiments: the donor of the immune system component or the donor of the target or both are immunized with an antigen. The antigen can be: an alloantigen; a
5 xenoantigen or an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte.

In preferred embodiments the donor of the components is: a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or mouse. In preferred embodiments, e.g., in the case of a wild-type donor, e.g., a human,
10 the manipulation that gives rise to Helios deregulation, e.g., an Helios lesion, can be introduced *in vitro*.

In preferred embodiments the donor of the target is: a human or nonhuman mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or mouse.

In preferred embodiments the reaction mixture includes an exogenously add
15 cytokine or antigen, e.g., a protein antigen.

In another aspect, the invention features a cell, or purified preparation of cells, which include an Helios transgene, or which otherwise misexpress an Helios gene. The cell preparation can consist of human or non human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include an Helios
20 transgene, e.g., a heterologous form of an Helios gene, e.g., a gene derived from humans (in the case of a non-human cell). The Helios transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous Helios gene, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which
25 are related to mutated or mis-expressed Helios alleles or for use in drug screening.

In another aspect, the invention features, a method of providing an antibody, e.g., a polyclonal or monoclonal antibody. The method includes:

providing a mammal, e.g., a mouse, having a cell which is Helios deregulated, e.g., which misexpresses, preferably underexpresses, Helios, e.g., a hematopoietic cell; and
30 isolating an antibody from the animal or from a cell derived from the animal, e.g., a hybridoma.

In preferred embodiments: the mammal is immunized with an antigen; the cell is exogenously supplied and one or both of the mammal, or the mammal which donates the cell, are immunized with an antigen. The antigen can be: an alloantigen; a xenoantigen; an autoantigen; a protein; or an antigen which gives rise to an anti-idiotypic lymphocyte. In
5 preferred embodiments the antigen is an autoantigen and the animal is not immunized.

In preferred embodiments: the mammal is a non-human mammal, e.g., a swine, a nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse.

In a preferred embodiment, the method further includes: allowing the Helios-misexpressing cell to divide and give rise to a proliferation-deregulated or antibody
10 producing cell, e.g., a lymphocyte.

In preferred embodiments: the proliferation-deregulated or antibody producing cell e.g., a lymphocyte, e.g., a transformed lymphocyte, is isolated from a lymphoma of the mammal.

In preferred embodiments: the mammal carries a mutation at the Helios gene, e.g., a
15 point mutation in or a deletion for all or part of the Helios gene, e.g., a mutation in the DNA binding region, e.g., a point mutation in, or a deletion for all or part of one or more of the four N-terminal zinc finger regions which mediate DNA binding of the Helios protein or for one or more of the two C terminal zinc finger regions which mediate dimerization of the Helios protein; the mammal is heterozygous or homozygous for an Helios transgene; the
20 mammal carries a mutation in the control region of the Helios gene.

In preferred embodiments the mammal, e.g., a mouse, is homozygous for null mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus.

In preferred embodiments the mammal, e.g., a mouse, is homozygous for null
25 mutations, e.g., it is homozygous for a deletion of the C terminal end of the protein, at the Helios locus and includes a mutation at Ikaros, e.g., a dominant negative mutation at Ikaros. Preferably the Ikaros mutation is heterozygous.

In preferred embodiments: the mammal carries homozygous mutations at the Helios gene, e.g., a point mutation or a deletion, which, inactivates one or both of transcriptional

activation or dimerization, which decreases the half life of the protein, or which inactivates one or both of the C terminal Zinc finger domains.

In preferred embodiments: the proliferation-deregulated or antibody producing cell is a homozygous mutant Helios cell e.g., a lymphocyte; the proliferation-deregulated or antibody producing lymphocyte is a B lymphocyte; the proliferation-deregulated or antibody producing cell is heterozygous or homozygous for an Helios transgene.

In preferred embodiments, the cell is a lymphocyte and is: a cell which secretes one or more anti-inflammatory cytokines; a cell which is antigen or idiotypic specific; a cell which produces, or over produces, antibodies, e.g., IgG, IgA, or IgE antibodies.

In a preferred embodiment: the Helios-misexpressing cell, e.g., a lymphocyte, is supplied exogenously to the mammal, e.g., to a homozygous wild-type Helios mammal or a mammal carrying a mutation at the Helios gene, e.g., a point mutation or a deletion for all or part of the Helios gene. If exogenously supplied, the cell can be a human or a nonhuman, e.g., a swine, nonhuman primate, e.g., a monkey, a goat, or a rodent, e.g., a rat or a mouse, lymphocyte. The exogenously supplied cell can be homozygous for null mutations, e.g., homozygous for a deletion of the C terminal end of the protein, at the Helios locus. The exogenously supplied cell can be homozygous for null mutations, e.g., homozygous for a deletion of the C terminal end of the protein, at the Helios locus and include a mutation at Ikaros, e.g., a dominant negative mutation at Ikaros. Preferably the Ikaros mutation is heterozygous.

In a preferred embodiment the method further comprises isolating one or more cells, e.g., lymphocytes, from the mammal, and allowing the cell or cells to proliferate into a clonal population of cells, e.g., lymphocytes.

In a preferred embodiment the method further comprises isolating one or more cells, e.g., lymphocytes, from the mammal, and allowing the cell or cells to proliferate into a clonal population of cells, e.g., lymphocytes, and isolating the antibody therefrom.

In preferred embodiments a cell from the animal is fused with a second cell to provide a hybridoma.

In preferred embodiments a cell from the animal is fused with a second cell to provide a hybridoma and the antibody is isolated from the hybridoma.

Cells, e.g., stem cells, treated by the method of the invention can be introduced into mammals, e.g., humans, non-human primates, or other mammals, e.g., rodents. In preferred embodiments the treatment is performed *ex vivo* and: the cell is autologous, e.g., it is returned to the same individual from which it was derived; the cell is allogeneic, i.e., it is from the same species as the mammal to which it is administered; the cell is xenogeneic, i.e., it is from a different species from the mammal to which it is administered.

An Helios-deregulated cell is a cell which has a mutant or misexpressed Helios gene, e.g., an inactivated Helios gene.

A hematopoietic cell, can be, e.g., stem cell, e.g., a totipotent or a pluripotent stem cell, or a descendent of a stem cell, e.g., a lymphocyte, e.g. a B lymphocyte or a T lymphocyte.

An Helios misexpressing animal, as used herein, is an animal in which one or more, and preferably substantially all, of the cells misexpress Helios.

A mutation at the Helios locus, as used herein, includes any mutation which alters the expression, structure, or activity of the Helios gene or its gene product. These include point mutations in and in particular deletions of all or part of the Helios coding region or its control region.

An exogenously supplied cell, tissue, or cell product, e.g., a cytokine, as used herein, is a cell, tissue, or a cell product which is derived from an animal other than the one to which is supplied or administered. It can be from the same species or from different species than the animal to which it is supplied.

A substantially homogenous population of two or more cells e.g., lymphocytes, as used herein, means a population of cells in which at least 50% of the cells, more preferably at least 70% of the cells, more preferably at least 80% of the cells, most preferably at least 90%, 95% or 99% of the subject cell type, e.g., lymphocytes. With respect to the Helios locus however, the cells can be all (+/-), all (-/-), or a mixture of (+/-) and (-/-) cells.

Culturing, as used herein, means contacting a cell or tissue with an environment which will support viability of the cell or tissue and which preferably supports proliferation of the cell or tissue.

A substantially purified preparation of cells, e.g., lymphocytes, as used herein, means a preparation of cells in which at least 50% of the cells, more preferably at least 70%

of the cells, more preferably at least 80% of the cells, most preferably at least 90%, 95% or 99% of the cells of the subject cell, e.g., are lymphocytes. With respect to the Helios locus however, the cells can be all (+/-), all (-/-), or a mixture of (+/-) and (-/-) cells.

Immunocompromised, as used herein, refers to a mammal in which at least one
5 aspect of the immune system functions below the levels observed in a wild-type mammal. The mammal can be immunocompromised by a chemical treatment, by irradiation, or by a genetic lesion resulting in, e.g., a nude, a beige, a nude-beige, or an Ikaros - phenotype. The mammal can also be immunocompromised by an acquired disorder, e.g., by a virus, e.g., HIV.

10 As used herein, an Helios transgene, is a transgene which includes all or part of an Helios coding sequence or regulatory sequence. The term also includes DNA sequences which when integrated into the genome disrupt or otherwise mutagenize the Helios locus. Helios transgenes sequences which when integrated result in a deletion of all or part of the Helios gene. Included are transgenes: which upon insertion result in the misexpression of
15 an endogenous Helios gene; which upon insertion result in an additional copy of an Helios gene in the cell; which upon insertion place a non-Helios gene under the control of an Helios regulatory region. Also included are transgenes: which include a copy of the Helios gene having a mutation, e.g., a deletion or other mutation which results in misexpression of the transgene (as compared with wild type); which include a functional copy of an Helios
20 gene (i.e., a sequence having at least 5% of a wild type activity, e.g., the ability to support the development of T, B, or NK cells); which include a functional (i.e., having at least 5% of a wild type activity, e.g., at least 5% of a wild type level of transcription) or nonfunctional (i.e., having less than 5% of a wild type activity, e.g., less than a 5% of a wild type level of transcription) Helios regulatory region which can (optionally) be operably
25 linked to a nucleic acid sequence which encodes a wild type or mutant Helios gene product or, a gene product other than an Helios gene product, e.g., a reporter gene, a toxin gene, or a gene which is to be expressed in a tissue or at a developmental stage at which Helios is expressed. Preferably, the transgene includes at least 10, 20, 30, 40 , 50, 100, 200, 500, 1,000, or 2,000 base pairs which have at least 50, 60, 70, 80, 90, 95, or 99 % homology with
30 a naturally occurring Helios sequence. Preferably, the transgene includes a deletion of all or some of exons 3 and 4, or a deletion for some or all of exon 7 of the Helios gene.

A "heterologous promoter", as used herein is a promoter which is not naturally associated with the Helios gene.

A "purified preparation" or a "substantially pure preparation" of an Helios polypeptide, or a fragment or analog thereof (or an Helios-Helios or Helios-Ikaros dimer), as used herein, means an Helios polypeptide, or a fragment or analog thereof (or an Helios-Helios or Helios-Ikaros dimer), which is free of one or more other proteins lipids, and nucleic acids with which the Helios polypeptide (or an Helios-Helios or Helios-Ikaros dimer) naturally occurs. Preferably, the polypeptide, or a fragment or analog thereof (or an Helios-Helios or Helios-Ikaros dimer), is also separated from substances which are used to purify it, e.g., antibodies or gel matrix, such as polyacrylamide. Preferably, the polypeptide, or a fragment or analog thereof (or an Helios-Helios or Helios-Ikaros dimer), constitutes at least 10, 20, 50 70, 80 or 95% dry weight of the purified preparation. Preferably, the preparation contains: sufficient polypeptide to allow protein sequencing; at least 1, 10, or 100 g of the polypeptide; at least 1, 10, or 100 mg of the polypeptide.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

A "treatment", as used herein, includes any therapeutic treatment, e.g., the administration of a therapeutic agent or substance, e.g., a drug.

A "substantially pure nucleic acid", e.g., a substantially pure DNA encoding an Helios polypeptide, is a nucleic acid which is one or both of: not immediately contiguous with one or both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the nucleic acid is derived; or which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other

DNA sequences. Substantially pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional Helios sequences.

"Homologous", as used herein, refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For example, if 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence (SEQ ID NO:2) is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence, then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100). For example, if 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous or have 60% sequence identity. BY way of example, the DNA sequences ATTGCC and TATGGC share 50% homology or sequence identity. Generally, a comparison is made when two sequences are aligned to give a maximum homology or sequence identity.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting

example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. Blast nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to the nucleic acids of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Programs which are equivalent in terms of the results they produce can be used.

The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

As used herein, the term "transgene" means a nucleic acid sequence (encoding, e.g., one or more Helios polypeptides or Helios-Ikaros dimers), which is partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of the selected

nucleic acid, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

As used herein, the term "transgenic cell" refers to a cell containing a transgene.

As used herein, a "transgenic animal" is any animal in which one or more, and preferably essentially all, of the cells of the animal includes a transgene. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

As used herein, the term "tissue-specific promoter" means a DNA sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence, such as the Helios and/or Ikaros gene, operably linked to the promoter, and which effects expression of the selected DNA sequence in specific cells of a tissue, such as lymphocytes. The term also covers so-called "leaky" promoters, which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well.

A polypeptide has Helios biological activity if it has one or more of the following properties: (1) the ability to react with an antibody, or antibody fragment, specific for (a) a wild type Helios polypeptide, (b) a naturally-occurring mutant Helios polypeptide, or (c) a fragment of either (a) or (b); (2) the ability to form Helios dimers, Helios/Aiolos, and/or Helios/Ikaros dimers; (3) the ability to modulate the development of hematopoietic stem cells; (4) the ability to stimulate transcription from a sequence; or (5) the ability to act as an antagonist or agonist of the activities recited in (1), (2), (3) or (4).

"Misexpression", as used herein, refers to a non-wild type pattern of Helios gene expression. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing, size, amino acid sequence, post-translational modification, stability, or biological activity of the expressed Helios and/or Ikaros polypeptide; a pattern of expression that

differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the Helios and/or Ikaros gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus; a ratio of Ikaros-Ikaros dimer to Helios-Helios dimer which differs from wild type; a ratio of Helios to Helios-Helios dimer, Ikaros-Ikaros dimer, or Ikaros-Helios dimer that differs from wild type; a ratio of Ikaros-Helios dimer to Helios, Ikaros, Helios-Helios dimer, or Ikaros-Ikaros dimer that differs from wild type.

As described herein, one aspect of the invention features a pure (or recombinant) nucleic acid which includes a nucleotide sequence encoding an Helios, and/or equivalents of such nucleic acids. The term "nucleic acid", as used herein, can include fragments and equivalents. The term "equivalent" refers to nucleotide sequences encoding functionally equivalent polypeptides or functionally equivalent polypeptides which, for example, retain the ability to react with an antibody specific for an Helios polypeptide. Equivalent nucleotide sequences will include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants, and will, therefore, include sequences that differ from the nucleotide sequence of Helios shown in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6 due to the degeneracy of the genetic code.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); *DNA Cloning*, Volumes I and II (D. N. Glover ed., 1985); *Oligonucleotide Synthesis* (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.), *Immunochemical Methods In Cell And*

Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

5 The Helios genes and polypeptides of the present invention are useful for studying, diagnosing and/or treating diseases associated with unwanted cell proliferation, e.g., leukemias or lymphomas. The gene (or fragment thereof) can be used to prepare antisense constructs capable of inhibiting expression of a mutant or wild type Helios gene encoding a polypeptide having an undesirable function. Alternatively, an Helios polypeptide can be
10 used to raise antibodies capable of detecting proteins or protein levels associated with abnormal cell proliferation or lymphocyte differentiation, e.g., T cell maturation. Furthermore, Helios peptides, antibodies or nucleic acids, can be used to identify the stage of lymphocyte differentiation, e.g., the stage of T cell differentiation.

 Other features and advantages of the invention will be apparent from the following
15 detailed description, and from the claims.

Brief description of the drawings

Figure 1 depicts an alignment of the predicted amino acid sequence of Helios with that of
20 Ikaros (SEQ ID NO:7) and Aiolos (SEQ ID NO:8). The four N-terminal zinc fingers (ZF1-4) comprising the DNA binding domain, the C-terminal zinc fingers (ZF5-6) that mediate protein dimerization and the conserved transcriptional activation domain (TAD) are outlined. Arrows indicate the conserved sequences to which the degenerate oligos Ik-1 (GEKPKF, Ik-F) and Ik-2 (YTIHMG, IK-R) were designed to clone the Helios gene.

25 *Figure 2* depicts a diagram of hemopoietic hierarchy of the progenitors and committed cells analyzed for Helios family gene expression.

Figure 3 depicts the mouse Helios-1 nucleotide (SEQ ID NO:1) and amino acid (SEQ ID
30 NO:2) sequences.

Figure 4 depicts the mouse Helios-2 nucleotide (SEQ ID NO:3) and amino acid (SEQ ID NO: 4) sequences.

Figure 5 depicts the human Helios-2 nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO: 6) sequences.

Figure 6 depicts an alignment of the nucleic acid sequence of mouse Helios with human Helios.

Figure 7 depicts an alignment of the amino acid sequence of mouse Helios with human Helios.

Detailed Description of the Invention

Gene Therapy

The gene constructs of the invention can also be used as a part of a gene therapy protocol to deliver nucleic acids encoding either an agonistic or antagonistic form of an Helios polypeptide. The invention features expression vectors for *in vivo* transfection and expression of an Helios polypeptide in particular cell types (e.g., dermal cells) so as to reconstitute the function of, enhance the function of, or alternatively, antagonize the function of an Helios polypeptide in a cell in which the polypeptide is expressed or misexpressed.

Expression constructs of Helios polypeptide, may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the Helios gene to cells *in vivo*. Approaches include insertion of the subject gene into viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaPO₄ precipitation carried out *in vivo*.

A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, e.g. a cDNA encoding an Helios polypeptide. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76, 271). A replication defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include ψ Crip, ψ Cre, ψ 2 and ψ Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see for example Eglitis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury et al. (1991) *Science* 254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S.

Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

Another viral gene delivery system useful in the present invention utilizes adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they are not capable of infecting nondividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld et al. (1992) cited *supra*). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al. cited *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267).

Yet another viral vector system useful for delivery of the subject Helios gene is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. *Curr. Topics in Micro. and Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski et al. (1989) *J. Virol.* 63:3822-3828; and McLaughlin et al. (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such

as that described in Tratschin et al. (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470; Tratschin et al. (1985) *Mol. Cell. Biol.* 4:2072-2081; Wondisford et al. (1988) *Mol. Endocrinol.* 2:32-39; Tratschin et al. (1984) *J. Virol.* 51:611-619; and Flotte et al. (1993) *J. Biol. Chem.* 268:3781-3790).

In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to cause expression of an Helios polypeptide in the tissue of a mammal, such as a human. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject Helios gene by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In a representative embodiment, a gene encoding an Helios polypeptide can be entrapped in liposomes bearing positive charges on their surface (e.g., lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue (Mizuno et al. (1992) *No Shinkei Geka* 20:547-551; PCT publication WO91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

In clinical settings, the gene delivery systems for the therapeutic Helios gene can be introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g. by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by stereotactic injection (e.g. Chen et al. (1994) *PNAS* 91: 3054-3057). In a preferred embodiment of the invention, the Helios gene is targeted to hematopoietic cells.

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery system can be produced in tact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Antisense Therapy

Another aspect of the invention relates to the use of the isolated nucleic acid in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize (e.g. bind) under cellular conditions, with the cellular mRNA and/or genomic DNA encoding an Helios polypeptide, or mutant thereof, so as to inhibit expression of the encoded protein, e.g. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

In one embodiment, the antisense construct binds to a naturally-occurring sequence of an Helios gene which, for example, is involved in expression of the gene. These sequences include, for example, start codons, stop codons, and RNA primer binding sites.

In another embodiment, the antisense construct binds to a nucleotide sequence which is not present in the wild type gene. For example, the antisense construct can bind to a region of an Helios gene which contains an insertion of an exogenous, non-wild type sequence. Alternatively, the antisense construct can bind to a region of an Helios gene which has undergone a deletion, thereby bringing two regions of the gene together which are not normally positioned together and which, together, create a non-wild type sequence.

When administered *in vivo* to a subject, antisense constructs which bind to non-wild type sequences provide the advantage of inhibiting the expression of mutant Helios gene, without inhibiting expression of any wild type Helios gene.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes a Helios polypeptide. Alternatively, the antisense construct is an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of an Helios gene. Such oligonucleotide probes are preferably modified oligonucleotide which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, and is therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van der Krol et al. (1988) *Biotechniques* 6:958-976; and Stein et al. (1988) *Cancer Res* 48:2659-2668.

Accordingly, the modified oligomers of the invention are useful in therapeutic, diagnostic, and research contexts. In therapeutic applications, the oligomers are utilized in a manner appropriate for antisense therapy in general. For such therapy, the oligomers of the invention can be formulated for a variety of loads of administration, including systemic and topical or localized administration. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous for injection, the oligomers of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the oligomers may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included in the invention.

The compounds can be administered orally, or by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art, and include, for example, for transmucosal administration bile salts and fusidic acid derivatives, and detergents. Transmucosal administration may be through nasal sprays or using suppositories. For oral administration, the oligomers are formulated into conventional oral administration forms such as capsules, tablets, and tonics. For topical administration,

the oligomers of the invention are formulated into ointments, salves, gels, or creams as known in the art.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the target DNA or RNA sequences to which they specifically bind.

The antisense constructs of the present invention, by antagonizing the expression of an Helios gene, can be used in the manipulation of tissue, both *in vivo* and in *ex vivo* tissue cultures.

10 *Transgenic Animals*

The invention includes transgenic animals which include cells (of that animal) which contain an Helios transgene and which preferably (though optionally) express (or misexpress) an endogenous or exogenous Helios gene in one or more cells in the animal.

The Helios transgene can encode a mutant Helios polypeptide. Such animals can be used as disease models or can be used to screen for agents effective at correcting the misexpression of Helios. Alternatively, the Helios transgene can encode the wild-type forms of the protein, or can encode homologs thereof, including both agonists and antagonists, as well as antisense constructs. In preferred embodiments, the expression of the transgene is restricted to specific subsets of cells, or tissues utilizing, for example, cis-acting sequences that control expression in the desired pattern. Tissue-specific regulatory sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory sequences. In preferred embodiments, the transgenic animal carries a "knockout" Helios gene, i.e., a deletion of all or a part of the Helios gene.

Genetic techniques which allow for the expression of transgenes, that are regulated *in vivo* via site-specific genetic manipulation, are known to those skilled in the art. For example, genetic systems are available which allow for the regulated expression of a recombinase that catalyzes the genetic recombination a target sequence. As used herein, the phrase "target sequence" refers to a nucleotide sequence that is genetically recombined by a recombinase. The target sequence is flanked by recombinase recognition sequences and is

generally either excised or inverted in cells expressing recombinase activity. Recombinase catalyzed recombination events can be designed such that recombination of the target sequence results in either the activation or repression of expression of the subject Helios gene. For example, excision of a target sequence which interferes with the expression of a recombinant Helios gene, such as one which encodes an agonistic homolog, can be designed to activate expression of that gene. This interference with expression of the protein can result from a variety of mechanisms, such as spatial separation of the Helios gene from the promoter element or an internal stop codon.

Moreover, the transgene can be made so that the coding sequence of the gene is flanked with recombinase recognition sequences and is initially transfected into cells in a 3' to 5' orientation with respect to the promoter element. In such an instance, inversion of the target sequence will reorient the subject gene by placing the 5' end of the coding sequence in an orientation with respect to the promoter element which allow for promoter driven transcriptional activation. See e.g., descriptions of the *cre/loxP* recombinase system of bacteriophage P1 (Lakso et al. (1992) *PNAS* 89:6232-6236; Orban et al. (1992) *PNAS* 89:6861-6865) or the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355; PCT publication WO 92/15694). Genetic recombination of the target sequence is dependent on expression of the Cre recombinase. Expression of the recombinase can be regulated by promoter elements which are subject to regulatory control, e.g., tissue-specific, developmental stage-specific, inducible or repressible by externally added agents. This regulated control will result in genetic recombination of the target sequence only in cells where recombinase expression is mediated by the promoter element. Thus, the activation expression of the recombinant Helios gene can be regulated via control of recombinase expression.

Similar conditional transgenes can be provided using prokaryotic promoter sequences which require prokaryotic proteins to be simultaneous expressed in order to facilitate expression of the transgene. Exemplary promoters and the corresponding trans-activating prokaryotic proteins are given in U.S. Patent No. 4,833,080. Moreover, expression of the conditional transgenes can be induced by gene therapy-like methods wherein a gene encoding the trans-activating protein, e.g. a recombinase or a prokaryotic protein, is delivered to the tissue and caused to be expressed, such as in a cell-type specific

manner. By this method, the Helios transgene could remain silent into adulthood until "turned on" by the introduction of the trans-activator.

Production of Fragments and Analogs

5 The inventor has provided the primary amino acid structure of an Helios polypeptide. Once an example of this core structure has been provided, one skilled in the art can alter the disclosed structure by producing fragments or analogs, and testing the newly produced structures for activity. Examples of prior art methods which allow the production and testing of fragments and analogs are discussed below. These, or analogous
10 methods can be used to make and screen fragments and analogs of an Helios polypeptide having at least one biological activity e.g., which react with an antibody (e.g., a monoclonal antibody) specific for an Helios polypeptide.

Generation of Fragments

15 Fragments of a protein can be produced in several ways, e.g., recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments.
20 Digestion with "end-nibbling" endonucleases can thus generate DNA's which encode an array of fragments. DNA's which encode fragments of a protein can also be generated by random shearing, restriction digestion or a combination of the above-discussed methods.

 Fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example,
25 peptides of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

Production of Altered DNA and Peptide Sequences: Random Methods

 Amino acid sequence variants of a protein can be prepared by random mutagenesis
30 of DNA which encodes a protein or a particular domain or region of a protein. Useful methods include PCR mutagenesis and saturation mutagenesis. A library of random amino

acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences. (Methods for screening proteins in a library of variants are elsewhere herein.)

5 PCR Mutagenesis

In PCR mutagenesis, reduced Taq polymerase fidelity is used to introduce random mutations into a cloned fragment of DNA (Leung et al., 1989, *Technique* 1:11-15). This is a very powerful and relatively rapid method of introducing random mutations. The DNA region to be mutagenized is amplified using the polymerase chain reaction (PCR) under
10 conditions that reduce the fidelity of DNA synthesis by Taq DNA polymerase, e.g., by using a dGTP/dATP ratio of five and adding Mn^{2+} to the PCR reaction. The pool of amplified DNA fragments are inserted into appropriate cloning vectors to provide random mutant libraries.

15 Saturation Mutagenesis

Saturation mutagenesis allows for the rapid introduction of a large number of single base substitutions into cloned DNA fragments (Mayers et al., 1985, *Science* 229:242). This technique includes generation of mutations, e.g., by chemical treatment or irradiation of single-stranded DNA *in vitro*, and synthesis of a complementary DNA strand. The mutation
20 frequency can be modulated by modulating the severity of the treatment, and essentially all possible base substitutions can be obtained. Because this procedure does not involve a genetic selection for mutant fragments both neutral substitutions, as well as those that alter function, are obtained. The distribution of point mutations is not biased toward conserved sequence elements.

25

Degenerate Oligonucleotides

A library of homologs can also be generated from a set of degenerate oligonucleotide sequences. Chemical synthesis of a degenerate sequences can be carried out in an automatic DNA synthesizer, and the synthetic genes then ligated into an
30 appropriate expression vector. The synthesis of degenerate oligonucleotides is known in the art (see for example, Narang, SA (1983) *Tetrahedron* 39:3; Itakura et al. (1981)

Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477. Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al. (1990) *Science* 249:386-390; Roberts et al. (1992) *PNAS* 89:2429-2433; Devlin et al. (1990) *Science* 249: 404-406; Cwirla et al. (1990) *PNAS* 87: 6378-6382; as well as U.S. Patents Nos. 5,223,409, 5,198,346, and 5,096,815).

Production of Altered DNA and Peptide Sequences: Methods for Directed Mutagenesis

Non-random or directed, mutagenesis techniques can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3.

Alanine Scanning Mutagenesis

Alanine scanning mutagenesis is a useful method for identification of certain residues or regions of the desired protein that are preferred locations or domains for mutagenesis, Cunningham and Wells (*Science* 244:1081-1085, 1989). In alanine scanning, a residue or group of target residues are identified (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine). Replacement of an amino acid can affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions are then refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the

target codon or region and the expressed desired protein subunit variants are screened for the optimal combination of desired activity.

Oligonucleotide-Mediated Mutagenesis

5 Oligonucleotide-mediated mutagenesis is a useful method for preparing substitution, deletion, and insertion variants of DNA, see, e.g., Adelman et al., (*DNA* 2:183, 1983). Briefly, the desired DNA is altered by hybridizing an oligonucleotide encoding a mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the desired protein.

10 After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the desired protein DNA. Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either

15 side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA*, 75: 5765[1978]).

Cassette Mutagenesis

20 Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene*, 34:315 [1985]). The starting material is a plasmid (or other vector) which includes the protein subunit DNA to be mutated. The codon(s) in the protein subunit DNA to be mutated are identified. There must be a unique

25 restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the desired protein subunit DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the

30 sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then

hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are comparable with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated desired protein subunit DNA sequence.

5

Combinatorial Mutagenesis

Combinatorial mutagenesis can also be used to generate mutants, e.g., a library of variants which is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For example, a mixture of synthetic oligonucleotides
10 can be enzymatically ligated into gene sequences such that the degenerate set of potential sequences are expressible as individual peptides, or alternatively, as a set of larger fusion proteins containing the set of degenerate sequences.

Primary High-Through-Put Methods for Screening Libraries of Peptide Fragments 15 or Homologs

Various techniques are known in the art for screening generated mutant gene products. Techniques for screening large gene libraries often include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the genes under conditions in which detection of a desired
20 activity, e.g., in this case, binding to an antibody specific for a Helios polypeptide. Each of the techniques described below is amenable to high through-put analysis for screening large numbers of sequences created, e.g., by random mutagenesis techniques.

Display Libraries

25 In one approach to screening assays, the candidate peptides are displayed on the surface of a cell or viral particle, and the ability of particular cells or viral particles to bind an appropriate receptor protein via the displayed product is detected in a "panning assay". For example, the gene library can be cloned into the gene for a surface membrane protein of a bacterial cell, and the resulting fusion protein detected by panning (Ladner et al., WO
30 88/06630; Fuchs et al. (1991) *Bio/Technology* 9:1370-1371; and Goward et al. (1992) *TIBS* 18:136-140). In a similar fashion, a detectably labeled ligand can be used to score for

potentially functional peptide homologs. Fluorescently labeled ligands, e.g., receptors, can be used to detect homolog which retain ligand-binding activity. The use of fluorescently labeled ligands, allows cells to be visually inspected and separated under a fluorescence microscope, or, where the morphology of the cell permits, to be separated by a
5 fluorescence-activated cell sorter.

A gene library can be expressed as a fusion protein on the surface of a viral particle. For instance, in the filamentous phage system, foreign peptide sequences can be expressed on the surface of infectious phage, thereby conferring two significant benefits. First, since these phage can be applied to affinity matrices at concentrations well over 10^{13} phage per
10 milliliter, a large number of phage can be screened at one time. Second, since each infectious phage displays a gene product on its surface, if a particular phage is recovered from an affinity matrix in low yield, the phage can be amplified by another round of infection. The group of almost identical *E. coli* filamentous phages M13, fd., and f1 are most often used in phage display libraries. Either of the phage gIII or gVIII coat proteins
15 can be used to generate fusion proteins without disrupting the ultimate packaging of the viral particle. Foreign epitopes can be expressed at the NH₂-terminal end of pIII and phage bearing such epitopes recovered from a large excess of phage lacking this epitope (Ladner et al. PCT publication WO 90/02909; Garrard et al., PCT publication WO 92/09690; Marks et al. (1992) *J. Biol. Chem.* 267:16007-16010; Griffiths et al. (1993) *EMBO J* 12:725-734;
20 Clackson et al. (1991) *Nature* 352:624-628; and Barbas et al. (1992) *PNAS* 89:4457-4461).

A common approach uses the maltose receptor of *E. coli* (the outer membrane protein, LamB) as a peptide fusion partner (Charbit et al. (1986) *EMBO* 5, 3029-3037). Oligonucleotides have been inserted into plasmids encoding the LamB gene to produce peptides fused into one of the extracellular loops of the protein. These peptides are
25 available for binding to ligands, e.g., to antibodies, and can elicit an immune response when the cells are administered to animals. Other cell surface proteins, e.g., OmpA (Schorr et al. (1991) *Vaccines* 9, pp. 387-392), PhoE (Agterberg, et al. (1990) *Gene* 88, 37-45), and PAL (Fuchs et al. (1991) *Bio/Tech* 9, 1369-1372), as well as large bacterial surface structures have served as vehicles for peptide display. Peptides can be fused to pilin, a protein which
30 polymerizes to form the pilus-a conduit for interbacterial exchange of genetic information (Thiry et al. (1989) *Appl. Environ. Microbiol.* 55, 984-993). Because of its role in

interacting with other cells, the pilus provides a useful support for the presentation of peptides to the extracellular environment. Another large surface structure used for peptide display is the bacterial motive organ, the flagellum. Fusion of peptides to the subunit protein flagellin offers a dense array of many peptides copies on the host cells (Kuwajima et al. (1988) *Bio/Tech.* 6, 1080-1083). Surface proteins of other bacterial species have also served as peptide fusion partners. Examples include the *Staphylococcus* protein A and the outer membrane protease IgA of *Neisseria* (Hansson et al. (1992) *J. Bacteriol.* 174, 4239-4245 and Klauser et al. (1990) *EMBO J.* 9, 1991-1999).

In the filamentous phage systems and the LamB system described above, the physical link between the peptide and its encoding DNA occurs by the containment of the DNA within a particle (cell or phage) that carries the peptide on its surface. Capturing the peptide captures the particle and the DNA within. An alternative scheme uses the DNA-binding protein LacI to form a link between peptide and DNA (Cull et al. (1992) *PNAS USA* 89:1865-1869). This system uses a plasmid containing the LacI gene with an oligonucleotide cloning site at its 3'-end. Under the controlled induction by arabinose, a LacI-peptide fusion protein is produced. This fusion retains the natural ability of LacI to bind to a short DNA sequence known as LacO operator (LacO). By installing two copies of LacO on the expression plasmid, the LacI-peptide fusion binds tightly to the plasmid that encoded it. Because the plasmids in each cell contain only a single oligonucleotide sequence and each cell expresses only a single peptide sequence, the peptides become specifically and stably associated with the DNA sequence that directed its synthesis. The cells of the library are gently lysed and the peptide-DNA complexes are exposed to a matrix of immobilized receptor to recover the complexes containing active peptides. The associated plasmid DNA is then reintroduced into cells for amplification and DNA sequencing to determine the identity of the peptide ligands. As a demonstration of the practical utility of the method, a large random library of dodecapeptides was made and selected on a monoclonal antibody raised against the opioid peptide dynorphin B. A cohort of peptides was recovered, all related by a consensus sequence corresponding to a six-residue portion of dynorphin B. (Cull et al. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89-1869)

This scheme, sometimes referred to as peptides-on-plasmids, differs in two important ways from the phage display methods. First, the peptides are attached to the C-

terminus of the fusion protein, resulting in the display of the library members as peptides having free carboxy termini. Both of the filamentous phage coat proteins, pIII and pVIII, are anchored to the phage through their C-termini, and the guest peptides are placed into the outward-extending N-terminal domains. In some designs, the phage-displayed peptides are presented right at the amino terminus of the fusion protein. (Cwirla, et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 6378-6382) A second difference is the set of biological biases affecting the population of peptides actually present in the libraries. The LacI fusion molecules are confined to the cytoplasm of the host cells. The phage coat fusions are exposed briefly to the cytoplasm during translation but are rapidly secreted through the inner membrane into the periplasmic compartment, remaining anchored in the membrane by their C-terminal hydrophobic domains, with the N-termini, containing the peptides, protruding into the periplasm while awaiting assembly into phage particles. The peptides in the LacI and phage libraries may differ significantly as a result of their exposure to different proteolytic activities. The phage coat proteins require transport across the inner membrane and signal peptidase processing as a prelude to incorporation into phage. Certain peptides exert a deleterious effect on these processes and are underrepresented in the libraries (Gallop et al. (1994) *J. Med. Chem.* 37(9):1233-1251). These particular biases are not a factor in the LacI display system.

The number of small peptides available in recombinant random libraries is enormous. Libraries of 10^7 - 10^9 independent clones are routinely prepared. Libraries as large as 10^{11} recombinants have been created, but this size approaches the practical limit for clone libraries. This limitation in library size occurs at the step of transforming the DNA containing randomized segments into the host bacterial cells. To circumvent this limitation, an *in vitro* system based on the display of nascent peptides in polysome complexes has recently been developed. This display library method has the potential of producing libraries 3-6 orders of magnitude larger than the currently available phage/phagemid or plasmid libraries. Furthermore, the construction of the libraries, expression of the peptides, and screening, is done in an entirely cell-free format.

In one application of this method (Gallop et al. (1994) *J. Med. Chem.* 37(9):1233-1251), a molecular DNA library encoding 10^{12} decapeptides was constructed and the library expressed in an *E. coli* S30 *in vitro* coupled transcription/translation system.

Conditions were chosen to stall the ribosomes on the mRNA, causing the accumulation of a substantial proportion of the RNA in polysomes and yielding complexes containing nascent peptides still linked to their encoding RNA. The polysomes are sufficiently robust to be affinity purified on immobilized receptors in much the same way as the more conventional recombinant peptide display libraries are screened. RNA from the bound complexes is recovered, converted to cDNA, and amplified by PCR to produce a template for the next round of synthesis and screening. The polysome display method can be coupled to the phage display system. Following several rounds of screening, cDNA from the enriched pool of polysomes was cloned into a phagemid vector. This vector serves as both a peptide expression vector, displaying peptides fused to the coat proteins, and as a DNA sequencing vector for peptide identification. By expressing the polysome-derived peptides on phage, one can either continue the affinity selection procedure in this format or assay the peptides on individual clones for binding activity in a phage ELISA, or for binding specificity in a completion phage ELISA (Barret, et al. (1992) *Anal. Biochem* 204,357-364). To identify the sequences of the active peptides one sequences the DNA produced by the phagemid host.

Secondary Screens

The high through-put assays described above can be followed by secondary screens in order to identify further biological activities which will, e.g., allow one skilled in the art to differentiate agonists from antagonists. The type of a secondary screen used will depend on the desired activity that needs to be tested. For example, an assay can be developed in which the ability to inhibit an interaction between a protein of interest and its respective ligand can be used to identify antagonists from a group of peptide fragments isolated through one of the primary screens described above.

Therefore, methods for generating fragments and analogs and testing them for activity are known in the art. Once the core sequence of a protein of interest is identified, such as the primary amino acid sequence of Helios polypeptide as disclosed herein, it is routine to perform for one skilled in the art to obtain analogs and fragments.

Peptide Analogs of Helios

Peptide analogs of an Helios polypeptide are preferably less than 400, 300, 200, 150, 130, 110, 90, 70 amino acids in length, preferably less than 50 amino acids in length, most preferably less than 30, 20 or 10 amino acids in length. In preferred embodiments, the peptide analogs of an Helios polypeptide are at least about 10, 20, 30, 50, 100 or 130 amino acids in length.

Peptide analogs of an Helios polypeptide have preferably at least about 60%, 70%, 74%, 80%, 85%, 90%, 95% or 99% homology or sequence similarity with the naturally occurring Helios polypeptide.

Peptide analogs of an Helios polypeptide differ from the naturally occurring Helios polypeptide by at least 1, 2, 5, 10 or 20 amino acid residues; preferably, however, they differ in less than 15, 10 or 5 amino acid residues from the naturally occurring Helios polypeptide.

Useful analogs of an Helios polypeptide can be agonists or antagonists. Antagonists of an Helios polypeptide can be molecules which form the Helios-Ikaros dimers but which lack some additional biological activity such as transcriptional activation of genes that control lymphocyte development. Helios antagonists and agonists are derivatives which can modulate, e.g., inhibit or promote, lymphocyte maturation and function.

A number of important functional Helios domains have been identified by the inventors. This body of knowledge provides guidance for one skilled in the art to make Helios analogs. One would expect nonconservative amino acid changes made in a domain to disrupt activities in which that domain is involved. Conservative amino acid changes, especially those outside the important functional domains, are less likely to modulate a change in activity. A discussion of conservative amino acid substitutions is provided herein.

The general structure of Helios and Ikaros proteins is very similar, and four blocks of sequence are particularly well conserved. The first block of conservation encodes the zinc finger modules contained in the Ik-1 isoform which mediate DNA binding of the Ikaros protein (Molnar et al. (1994) *Mol. Cell. Biol.* 14 8292-8303). The second block of conservation has not been characterized functionally.

The third block of conservation a highly conserved 81 amino acid sequence which has been shown to mediate transcriptional activity of the Ikaros proteins. This activation

domain of Ikaros is composed of a stretch of acidic amino acids followed by a stretch of hydrophobic residues, both of which are required for its full activation potential. This domain from Ikaros alone or the full length Ikaros protein confers transcriptional activity of a fusion protein with the LexA DNA binding domain. This example shows that the homologous domain in Helios is also a transcriptional activation domain in yeast and mammalian cells and that the Helios transcriptional activation domain provides stronger transcriptional activity than the homologous domain from Ikaros in mammalian cells. The results show that the 232 C-terminal amino acids of Helios is capable of conferring transcriptional activation in yeast cells. No activity was detected with the 149 most C-terminal amino acids of Helios, which do not contain the conserved domain.

The fourth block of conservation corresponds to the zinc fingers which mediate dimerization. A C-terminal 149 amino acids of Helios which contain the two terminal zinc finger domains mediate protein dimerization.

15 *Antibodies*

The invention also includes antibodies specifically reactive with a subject Helios polypeptide or Helios-Ikaros dimers. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of the subject Helios polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies. In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of the Helios-Ikaros dimers or Helios polypeptide of the invention, e.g. antigenic determinants of a polypeptide of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

30 The term "antibody", as used herein, intended to include fragments thereof which are also specifically reactive with an Helios polypeptide or Helios-Ikaros dimers.

Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

5 Both monoclonal and polyclonal antibodies (Ab) directed against Helios-Ikaros dimers or Helios polypeptides, or fragments or analogs thereof, and antibody fragments such as Fab' and $F(ab')_2$, can be used to block the action of an Helios and/or Ikaros polypeptide and allow the study of the role of an Helios polypeptide of the present invention.

10 Antibodies which specifically bind Helios-Ikaros dimers or Helios polypeptide epitopes can also be used in immunohistochemical staining of tissue samples in order to evaluate the abundance and pattern of expression of Helios-Ikaros dimer or Helios polypeptide. Anti-Helios polypeptide antibodies can be used diagnostically in immuno-precipitation and immuno-blotting to detect and evaluate wild type or mutant Helios
15 polypeptide levels in tissue or bodily fluid as part of a clinical testing procedure. Likewise, the ability to monitor Helios-Ikaros dimer or Helios polypeptide levels in an individual can allow determination of the efficacy of a given treatment regimen for an individual afflicted with disorders associated with modulation of lymphocyte differentiation and/or proliferation. The level of an Helios-Ikaros dimer or Helios polypeptide can be measured in
20 tissue, such as produced by biopsy.

Another application of anti-Helios antibodies of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as gt11, gt18-23, ZAP, and ORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins.
25 For instance, gt11 will produce fusion proteins whose amino termini consist of β -galactosidase amino acid sequences and whose carboxy termini consist of a foreign polypeptide. Antigenic epitopes of a subject Helios polypeptide can then be detected with antibodies, as, for example, reacting nitrocellulose filters lifted from infected plates with anti-Helios polypeptide antibodies. Phage, scored by this assay, can then be isolated from
30 the infected plate. Thus, the presence of Helios homologs can be detected and cloned from

other animals, and alternate isoforms (including splicing variants) can be detected and cloned from human sources.

Drug Screening Assays

5 By making available purified and recombinant-Helios polypeptides, the present invention provides assays which can be used to screen for drugs which are either agonists or antagonists of the normal cellular function, in this case, of the subject Helios polypeptide. In one embodiment, the assay evaluates the ability of a compound to modulate binding between an Helios polypeptide and a naturally occurring ligand, e.g., an antibody specific
10 for a Helios polypeptide or an Ikaros polypeptide. A variety of assay formats will suffice and, in light of the present inventions, will be comprehended by skilled artisan.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free
15 systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused
20 primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with other proteins or change in enzymatic properties of the molecular target.

Other Embodiments

25 Included in the invention are: allelic variations; natural mutants; induced mutants; proteins encoded by DNA that hybridizes under high or low stringency conditions to a nucleic acids which encode polypeptides of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6 (for definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference); and,
30 polypeptides specifically bound by antisera to an Helios polypeptide.

Nucleic acids and polypeptides of the invention includes those that differ from the sequences disclosed herein by virtue of sequencing errors in the disclosed sequences.

Also included in the invention is a composition which includes an Helios polypeptide, e.g., an Helios/Helios dimer or an Helios/Ikaros peptide, and one or more
5 additional components, e.g., a carrier, diluent, or solvent. The additional component can be one which renders the composition useful for *in vitro*, *in vivo*, pharmaceutical, or veterinary use. Examples of *in vitro* use are binding studies. Examples of *in vivo* use are the induction of antibodies.

The invention also includes fragments, preferably biologically active fragments, or
10 analogs of an Helios polypeptide. A biologically active fragment or analog is one having any *in vivo* or *in vitro* activity which is characteristic of the Helios polypeptide shown in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or of other naturally occurring Helios polypeptides, e.g., one or more of the biological activities described above. Especially preferred are fragments which exist *in vivo*, e.g., fragments which arise from post
15 transcriptional processing or which arise from translation of alternatively spliced RNA's. Fragments include those expressed in native or endogenous cells, e.g., as a result of post-translational processing, e.g., as the result of the removal of an amino-terminal signal sequence, as well as those made in expression systems, e.g., in CHO cells. Because peptides, such as an Helios polypeptide, often exhibit a range of physiological properties
20 and because such properties may be attributable to different portions of the molecule, a useful Helios polypeptide fragment or Helios polypeptide analog is one which exhibits a biological activity in any biological assay for Helios polypeptide activity. Most preferably the fragment or analog possesses 10%, preferably 40%, or at least 90% of the activity of an Helios polypeptide (SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6), in any *in vivo* or *in*
25 *vitro* Helios polypeptide activity assay.

Analogous can differ from a naturally occurring Helios polypeptide in amino acid sequence or in ways that do not involve sequence, or both. Non-sequence modifications include *in vivo* or *in vitro* chemical derivatization of an Helios polypeptide. Non-sequence modifications include changes in acetylation, methylation, phosphorylation, carboxylation,
30 or glycosylation.

Preferred analogs include an Helios polypeptide (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the Helios polypeptide biological activity.

- 5 Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative substitutions can be taken from the table below.

10

TABLE 1

CONSERVATIVE AMINO ACID REPLACEMENTS

| For Amino Acid | Code | Replace with any of |
|----------------|------|---|
| Alanine | A | D-Ala, Gly, beta-Ala, L-Cys, D-Cys |
| Arginine | R | D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn |
| Asparagine | N | D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln |
| Aspartic Acid | D | D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln |
| Cysteine | C | D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr |

| | | |
|---------------|---|--|
| Glutamine | Q | D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp |
| Glutamic Acid | E | D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln |
| Glycine | G | Ala, D-Ala, Pro, D-Pro, β -Ala Acp |
| Isoleucine | I | D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met |
| Leucine | L | D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met |
| Lysine | K | D-Lys, Arg, D-Arg, homo-Arg, D- homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn |
| Methionine | M | D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val |
| Phenylalanine | F | D-Phe, Tyr, D-Thr, L-Dopa, His, D- His, Trp, D-Trp, Trans-3,4, or 5- phenylproline, cis-3,4, or 5-phenylproline |
| Proline | P | D-Pro, L-I-thioazolidine-4- carboxylic acid, D-or L-1- oxazolidine-4-carboxylic acid |

| | | |
|-----------|---|--|
| Serine | S | D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D- Cys |
| Threonine | T | D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val |
| Tyrosine | Y | D-Tyr, Phe, D-Phe, L-Dopa, His, D- His |
| Valine | V | D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met |

Other analogs within the invention are those with modifications which increase peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids; and cyclic analogs.

As used herein, the term "fragment", as applied to an Helios polypeptide analog, will ordinarily be at least about 20 residues, more typically at least about 40 residues, preferably at least about 60 residues in length. Fragments of an Helios polypeptide can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of an Helios polypeptide can be assessed by methods known to those skilled in the art, as described herein. Also included are Helios polypeptides containing residues that are not required for biological activity of the peptide or that result from alternative mRNA splicing or alternative protein processing events.

In order to obtain an Helios polypeptide, an Helios polypeptide-encoding DNA can be introduced into an expression vector, the vector introduced into a cell suitable for expression of the desired protein, and the peptide recovered and purified, by prior art methods. Antibodies to the peptides and proteins can be made by immunizing an animal,

e.g., a rabbit or mouse, and recovering anti-Helios polypeptide antibodies by prior art methods.

Examples:

5 Identification of Helios, a novel Ikaros-related gene

In order, to identify a novel Ikaros-related factor a PCR-based approach was used. Degenerate primers, GEKPFK and YTIHMG, encoding conserved sequences in the Ikaros N-terminal (Ik-F) and C-terminal zinc finger (Ik-R) domains (Turpen et al., Immunity, 7:325-334, 1997) were used to amplify cDNAs generated from the spleen of Aiolos mutant mice. A PCR product of the expected 980 base pair size was cloned and shown to have unique DNA sequence with homology to the Ikaros gene. Full length coding sequence was obtained by RACE PCR using nested specific internal primers. Nested gene specific primers were as follows: 5'-RACE R51: GGGTGAAGGCCTCAGGT (SEQ ID NO:9) and R52: CCATCATATGAGACTGCATCAGCTCCAGCCTCC (SEQ ID NO:10); 3'-RACE R31: 15 GGAGGCTGAGCTGATGCACTCTCATATGATGG (SEQ ID NO:11) and R32: CACCTACCTTGGAGCTGAGGCCCTTCACCC (SEQ ID NO:12). The RACE PCR was performed using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA) and TaKaRa LA Taq DNA polymerase (Takara Shuzo, Shiga, Japan). The amplification conditions were 1.5 min. at 95°C for 1 cycle, 20 seconds at 98°C and 2.5 minutes at 72°C for 5 cycles, 20 seconds at 98°C and 2.5 minutes at 70°C for 5 cycles, 30 seconds at 98°C and 2.5 minutes at 68°C for 32 cycles, and 1 cycle of 10 minutes at 72°C. A second round of amplification using nested primers was performed using a portion of the first product as template. The second amplification was 1.5 minutes at 95°C for 1 cycle, 20 seconds at 98°C and 2.5 minutes at 68°C for 20 cycles, followed by 72°C for 10 minutes for 1 cycle. 5' and 25 3' products were cloned into the pGEM-T Easy vector (Promega, Madison, Wisconsin) and sequenced PCR analysis of Helios expression in hematopoietic cells using various combination of specific 5' and 3' primer pairs routinely yielded two bands. These two bands were cloned and sequenced to show that the two alternatively spliced transcripts differed in the presence of sequence encoding the first N-terminal zinc finger.

30 The encoded protein, designated Helios, shows a high degree of conservation to Ikaros and Aiolos (73% and 67% similarity overall, respectively) (Figure 1). The three

proteins are nearly identical throughout the N-terminal zinc finger DNA-binding domain. There is a 93% identity between Helios and Ikaros from the first through the fourth zinc fingers and 88% identity between the same regions of Helios and Aiolos. The protein dimerization domain, comprising the C-terminal zinc fingers is 86% identical between Helios and Ikaros and 75% identical between Helios and Aiolos. In a third region, that contains the transcriptional activation domain, Helios shares 68% similarity to Ikaros and 70% identity to Aiolos. As mentioned above, two alternatively spliced forms of Helios were identified by PCR from thymus cDNA. Sequence analysis of the two Helios isoforms revealed that they encode products that differ in the number of N-terminal zinc fingers. The full length isoform (Hel-1) is analogous to Ikaros isoform Ik-1 in that it contains all four DNA-binding zinc fingers. The second isoform (Hel-2) is similar to Ik-2 in that it is missing zinc finger 1, although the exon removed to generate Ik-2 includes additional sequence N-terminal to the zinc finger that is retained in the Hel-2 isoform. PCR analysis using various combinations of primer pairs revealed no other isoforms that migrate at approximately 64 and 66 kDa, as described below. No other proteins are detected by Western blot analysis of thymocyte nuclear extracts using an affinity purified polyclonal antibody against Helios. The strong conservation of the N-terminal zinc finger motifs of Hel-1 and Hel-2 with Ikaros isoforms Ik-1 and Ik-2 predicts that they will display similar affinities and DNA binding specificities.

5' and 3' RACE strategies were used to clone the ends of the human Helios cDNA after cloning of the internal section of this message using degenerate primers from the 5' and 3' zinc finger regions. The 3' untranslated sequence extends for an additional 3 kb. The human clone encodes a protein which is identical to mouse Helios. The nucleotide and inferred protein sequences of mouse and human Helios were compared using the GCG Bestfit program.

Expression of Helios during embryogenesis

The expression of Helios during mouse embryogenesis was examined by in situ hybridization. Ikaros expression was analyzed in an adjacent section at each stage for comparison. In situ hybridization, including embryo preparation, probe synthesis and in

situ hybridization, was carried out essentially as described [Ikeda, Dev. Dynamics, 20:318-329, 1996]. Four micrometer sections were prepared from embryonic days 8, 11, 13 and 16 and were hybridized with single stranded [33P]UTP labeled antisense RNA probes specific to each gene. Slides were exposed for 5 weeks, stained with hematoxylin and eosin and analyzed with both bright and dark field microscopy.

Helios was found to be expressed in all hematopoietic centers of the developing embryo. The blood islands of the yolk sac constituted the first site of embryonic hematopoiesis. Helios and Ikaros were expressed in this extraembryonic site at day 8 of gestation. However, by day 11, Helios expression was significantly decreased, while Ikaros expression was maintained through embryonic day 13 in this region. Both Helios and Ikaros were expressed in the liver at day 11; however, Helios mRNA was present in a subset of cells in this tissue. Throughout hematopoietic development, Helios expression in the liver was detected in a small number of scattered cells. In contrast, Ikaros was expressed at high levels in most of the cells present in this tissue during mid to late gestation. In the thymus, Helios was first detected at low levels at embryonic day 13, while Ikaros expression was readily detected in this site two days earlier. By day 16, Helios was expressed at high levels toward the center of the thymus, a region where early progenitors enter from the vasculature. In contrast, Ikaros was detected in most thymocytes. This pattern of Helios expression was maintained in the postnatal thymus. Helios was also detected in a small subset of cells within the spleen of the adult. Within the splenic germinal centers of an immunized animal, a small number of cells expressed moderate levels of Helios, while Ikaros was present at high levels throughout these centers. Their localization suggested that these may be CD4⁺TH₂ cells.

Outside of the hematopoietic system, Helios expression was high in a number of epithelial tissues. These include the endoderm lining the gut, the tubules of the kidney, the lining of the respiratory tract and olfactory epithelium. During late gestation high levels of Helios expression were detected in the salivary glands and ducts.

The expression of Helios in adult tissues was examined by Northern blot analysis of polyA⁺ selected RNAs using the region between the N- and C-terminal zinc fingers of Helios as a probe. Northern blot analysis and RT-PCR were carried out essentially as follows. A 980 bp cDNA was used as a probe for Northern analysis. This probe did not

cross react with Ikaros or Aiolos, which yield transcripts of distinct sizes. The blot had previously been screen with a GAPDH probe to confirm equivalent loading of RNA samples. Northern results showed that a transcript of approximately 8 kb was detected in thymus. At various times during embryogenesis, Helios was expressed in the lung, liver, kidney and brain; however, Helios mRNA was not detected by Northern analysis in these tissues in the adult. The Helios probe did not cross react with either Ikaros or Aiolos that encode more abundant messages of distinct sizes in the thymus and spleen.

Expression of Helios in Hematopoietic Subpopulations

The expression of Ikaros gene family members in sorted hematopoietic and lymphoid progenitors of the adult was examined by RT-PCR using specific primer pairs for Helios, Ikaros or Aiolos. RT-PCR conditions and Ikaros and Aiolos primers were carried out. HPRT primers (for.TGGCCCTCTGTGGTGCTCAAG (SEQ ID NO:13); Rev:CACAGGACTAGAACACCTGC (SEQ ID NO:14) were used as a control for RNA recovery. For analysis of Helios expression in hematopoietic cells, the following primer pairs were used: Forward (2F): GAACACGCCAATATGGCC (SEQ ID NO:15) (nucleotides 60-78 of Helios cDNA) and Reverse (8R): GGCCTTGGTAGCATCCAAAGC (SEQ ID NO:16) (nucleotides 1327-47 of Helios cDNA). For PCRs, primers 125F: AGAATGTCAGCATGGAGGCT (SEQ ID NO:17) (nucleotides 707-726) and 8R were used for amplification. This forward primer is downstream of the region encoding the first zinc finger and therefore, only amplifies one Helios isoform. In all cases, the annealing temperature was 60°C and amplification was determined to be in the linear range. For bone marrow derived progenitor populations where cells were limiting in number, cDNA from 50 cells equivalents was amplified for 32 cycles. For thymocyte precursors, amplification was done 1000 cell equivalents for 26 cycles for each primer pair, For other samples, e.g., Ikaros, 25 cycles were done and for Helios and Aiolos 28 cycles were done.

The subsets of hemo-lymphoid populations used for these studies and their ontogeny are diagrammed in Figure 2. Stem cell population (ckit⁺ Sca-1⁺, lineage⁻), early progenitors (ckit⁺ Sca-1⁻, lineage⁻ and ckit⁺SCA-1⁺Sca2⁺, lineage⁻) were purified from the bone marrow of wild type mice . Lineage committed erythroid (Ter119⁺), pre B (B220⁺), granulocyte

(Mac1⁺, GR⁺) monocyte/macrophage (Mac1⁺, GR⁻) populations were purified from bone marrow of wild type mice using antibodies to cell surface markers, magnetic secondary antibodies and separated using a MACS magnetic separation column. Pro B cells were B220⁺ sorted from the bone marrow of Rag^{-/-} mice, mature B cells were B220⁺ from the spleens of wild type mice. Splenocyte from Rag^{-/-} mice were depleted of red cells and used an enriched source of NK Thymic and splenic dendritic cells were purified. Double positive (CD4⁺CD8⁺) and single positive (CD4⁺ or CD8⁺) were sorted from wild type thymus and soluble negative (CD4⁻CD8⁻) were obtained from thymocyte of Rag^{-/-} mice that are arrested at this state of differentiation. Developmental stages of double thymocyte (CD4^{lo}, ckit⁺CD25⁺, ckit⁻CD25⁺, ckit⁻, CD25⁻) were sorted to 98-99% purity.

Helios mRNA was detected in the bone marrow progenitor population that was highly enriched for stem cell activity (ckit⁺/Sca-1⁺lineage⁻) and was also present in hematopoietic progenitors with more restricted lymphoid or erythro-myeloid potential (ckit⁺/Sca-1⁺/Sca-2⁺ and ckit⁺/Sca-1⁻/Sca-2⁻ respectively). Ikaros displays a similar pattern of expression in these hematopoietic progenitor populations whereas Aiolos was detected only in the progenitors that were more committed to lymphoid development (ckit⁺/Sca-1⁺/Sca-2⁺).

Helios was expressed in definitive erythroid precursors (ter119⁺) and very low levels of Helios mRNA are present within the monocyte (mac1⁺GR⁻) and granulocyte (Mac1⁺GR⁺) population in the adult bone marrow. Ikaros, but not Aiolos, was detected at low levels in all three of these cell types. Helios was present at low levels in pro-B cells (CD45R⁺/CD43⁺), and decreases as they progress to pre-B cells (CD45R⁺/IgM⁺). In contrast, Aiolos expression was low in pro-B cells and dramatically increases in pre-B and mature B cells.

As HSCs differentiate along the myeloerythroid and B lymphoid lineages, Helios expression was diminished. However, Helios was present at varying levels in all T cell subsets analyzed. The earliest lymphoid progenitors entering the thymus are CD4^{lo} (and ckit⁺) and are not necessarily committed to the T cell lineage. Helios and Ikaros are both detected in these earliest lymphoid progenitors. An increase in Helios was apparent during the progressive transition to the ckit⁺CD25⁺, and then ckit⁻CD25⁺, where Aiolos was first detected. A marked increase in Aiolos levels was observed at the next stage (ckit⁻CD25⁻),

while Helios expression decreases. Ikaros levels remain constant during these early stages of T-cell differentiation. For comparison, the expression of these genes in RNA from total thymocyte populations of wild type and Rag^{-/-} mice was done. In Rag^{-/-} mice the majority of thymocytes are at the ckit⁺CD25⁺ stage where T cell development was blocked, while in a wild type thymus, the majority of cells are at the later, double positive (CD4⁺CD8⁺) stage. Helios mRNA increases as T cells progress from the CD4⁻CD8⁻ double negative to the CD4⁺CD8⁺ double positive stage and declines as these become single positive (CD4⁺ or CD8⁺) thymocytes. Peripheral T cells have lower expression of Helios than immature thymocytes with the highest levels detected among $\gamma\delta$ T cells of the skin (V $\gamma\delta$) and the gut (IEL). Ikaros and Aiolos are present in these T cell populations but Aiolos was not detected in the fetally derived skin $\gamma\delta$ T cells. All three genes are expressed in NK cells. The lymphoid derived thymic dendritic cells (DC) as well the splenic CD8⁺ and CD8⁻ dendritic subsets express very low levels of Helios. Ikaros was present in all three populations, but was highest in the splenic CD8-DC subset. Among the DC subpopulations, Aiolos was also highest in the splenic DC8-DCs.

The expression of two Helios isoforms was routinely detected by PCR using a 5' primer preceding the first zinc finger. These isoforms correspond to the Hel-1 and Hel-2 cDNAs and are expressed at roughly equivalent levels in all cell types tested. As described previously, no significant difference in the ratio of Ikaros isoforms in different hematopoietic populations can be detected under our conditions, where amplification was determined to be within the linear range. In all hematopoietic cell types analyzed, Ik-1 and Ik-2 were expressed in highest abundance, while Ik-4 and 5 were expressed at low levels. A faint band corresponding to Ik-6 was also detected in all populations tested.

During hematopoietic development, Helios, Ikaros and Aiolos have overlapping but distinct patterns of expression. The differential patterns of expression of these three factors within the hematopoietic system may underscore their specific regulatory roles during differentiation.

The expression of Helios at the sites where HSCs arise suggest that this gene is an important regulators of the earlier stages of hematopoietic development. Hematopoietic progenitors accumulate in the yolk sac at day 8 and the fetal liver in day 11. Both Ikaros and Helios are expressed in similar numbers of cells in these regions at early states. As

gestation continues these sites are increasingly populated by more committed erythroid progenitors as well. While Ikaros expression increases in both sites, Helios was only expressed in a limited number of cells. This may reflect its preferential expression in the less committed hematopoietic progenitors in the embryo. The expression of Helios in sorted hematopoietic cells in the adult supports this interpretation. Helios is expressed in adult HSC's but its expression decreases in the maturing erythroid, macrophage and B-lymphocyte lineages. Helios expression peaks in the early stages of T-cell development and decrease as T cells mature in the thymus and are exported to the periphery. Significant levels of Helios are maintained in only a small subset of mature T-cells. Upon immunization, Helios is detected in a very small number of cells in germinal centers of the spleen. When compared with that of Ikaros and Aiolos, this profile of Helios expression suggests that transcriptional complexes including Ikaros and Helios will predominate in the earlier stages of hematopoiesis. This combination may be important for the self-renewing capacity of early progenitors that is compromised in the Ikaros DN homozygous mice. The increasing expression of Aiolos and Ikaros as development proceeds may lead to complexes that promote lineage progression and differentiation.

While Ikaros and Aiolos are predominately expressed in the hematopoietic system, Helios is also expressed elsewhere in the embryo. Based on this observation, it likely that the Ikaros gene family regulates lineage progression on other tissues as well. The dynamic expression of Helios in the embryo is consistent with such a role. Mutational analysis of the Helios gene will help to dissect its role in regulating progenitor development in the hematopoietic system and elsewhere in the embryo.

Helios forms homodimers and heterodimerizes with Ikaros and Aiolos

The C-terminal zinc fingers of Ikaros and Aiolos, shown to mediate their homo- and heterodimerization, are highly conserved in Helios. Helios-specific polyclonal antibodies were generated to study the interactions between the Helios isoforms and the Ikaros and Aiolos proteins. Generation of Helios-specific polyclonal antibodies was carried out as follows. The region between the N- and C-terminal zinc fingers of Helios was amplified by PCR and cloned in frame into a pRSET vector (Invitrogen, Carlsbad, CA). The protein was

expressed in BL21 E.coli and denatured protein was purified on a nickel affinity column as recommended by the manufacturer (Invitrogen, Carlsbad, CA). Rabbit polyclonal antibodies raised to this protein were affinity purified by pH elution. Specificity of this antibody for Helios and not other Ikaros homologs was confirmed by Western blot analysis of protein extracts from transfected 293T cells and by immunofluorescence of transfected cells. For Western analysis, protein lysates were taken up in 1X Laemmli sample buffer, heated at 95°C for 15 minutes and resolved on a 10% SDS-PAGE gel. Resolved proteins were transferred to an Immobilon-P membrane that was probed with the affinity purified polyclonal Helios antibodies (1/500 dilution in PBS, 0.05% TWEEN-20). To detect Helios in primary cells, signal was amplified by incubation of the filter with a 1/5000 dilution of biotinylated Goat α -rabbit antibody followed by the same dilution of peroxidase coupled streptavidin (Jackson labs). The ECL kit (Amersham, Uppsala, Sweden) was used for detection.

The antibody generated against Helios recognized the two Helios isoforms in thymocyte nuclear extracts from wild type, Ikaros null, and Ikaros DN \pm mutant mice. The Helios isoforms detected in thymocytes were approximately 64 and 68 kDa, and co-migrated with the proteins produced by the Hel-1 and Hel-2 cDNAs when co-expressed in the epithelial cell line 293T.

To determine whether Helios physically interacts with Ikaros in primary cells, we used cell lysates from the thymuses of mice transgenic for an epitope-tagged (FLAG) tagged Ik-7 isoform expressed from the CD2 minigene. Ik-7 was the predominant isoform produced by the Ikaros DN mutant locus and lacks the DNA binding domain, but has intact C-terminal dimerization zinc finger motifs. Complexes were immunoprecipitated from thymic whole cell lysates using a mouse monoclonal antibody specific for the FLAG epitope. Western blot analysis using the Helios polyclonal antibody revealed the presence of both Helios isoforms in the immunoprecipitated complexes. Thus, the Ikaros DN protein formed a stable protein complex with Helios protein isoforms and may interfere with their normal activity in vivo.

To examine more closely the ability of Helios isoforms Hel-1 and Hel-2 to form dimers with self, as well as with Ikaros and Aiolos, these factors were transiently expressed in 293 T cells in pairwise combinations. Transient expression of Ikaros and Aiolos in 293T

cells was carried out as follows. Full length Hel-1 or the Hel-2 isoforms were amplified by PCR from thymocyte cDNA using primers generated to the 5' or 3' ends (5' AATTGAATTCATGCACTGCACTTTGACTATGG (SEQ ID NO:18) and 3'R: TTTTCCTTTTGCGGCCGCATGTCGCCATCCGAGGGAAGG SEQ ID NO:19) and
5 cloned into the CDM8 mammalian expression vector between the EcoRI and NotI sites (CDM8-Hel-1, CDM8-Hel-2). Additional constructs were generated having the FLAG or hemagglutinin (HA) tags (FLAG-Hel-1, FLAG-HE1-2, HA-HE1, HA-HE1-2). The clones were sequenced to confirm no mutations were introduced and that they were in frame with epitope tags. 293T cells were transfected with 10µg of each cDNA. After two days, cells
10 from each 10 cm plate were harvested in 0.5 ml lysis buffer, 10 µl of extract was used to confirm expression of each protein by Western blot analysis, and 100 µl of extract precleared with protein G-agarose followed by immunoprecipitation with anti-FLAG M5 affinity gel. After washing, beads were resuspended in 2X Laemmli sample buffer and incubated for 15 minutes at 95°C. The beads were spun down and one third of the
15 supernatant was resolved on a 10% SDS-PAGE gel. Western blot analysis was carried out as described above except that for 293T extracts, incubation with affinity purified polyclonal antibodies specific for Ikaros or Helios was followed by incubation with peroxidase coupled Goat-α-rabbit secondary antibody. For immunoprecipitation from primary cells, thymocyte or splenocyte were obtained from transgenic mice expressing the
20 FLAG-tagged dominant negative mutant Ikaros isoform Ik-7 from the CD2 minigene. Cells were harvested and washed in PBS/2% FCS. Protein extracts were prepared by lysis of 1×10^7 cells per 100 µl lysis buffer.

As mentioned above, to determine whether Helios isoforms Hel-1 and Hel-2 can form dimers with self, as well as with Ikaros and Aiolos, these factors were transiently
25 expressed in 293 T cells in pairwise combinations. One protein in each expressed pair was epitope tagged (FLAG). After two days, cell lysates were prepared and Western blot analysis confirmed protein expression using antibodies specific for each of the Helios, Ikaros and Aiolos proteins. An antibody to the epitope tag (anti-FLAG) was used to immunoprecipitate complexes from 293T cell lysates, and precipitated complexes were
30 analyzed for protein interactions using Ikaros or Helios specific antibodies. The anti FLAG antibody co-precipitates both FLAG-Hel-1 and Hel-2, demonstrating that the two isoforms

can dimerize. A similar strategy was used to study Helios, Ikaros and Aiolos interactions. FLAG-Hel-1 or FLAG-Hel-2 were co-expressed with Ik-1 . The anti-FLAG antibody brought down IK-1 in an immunoprecipitated complex in both cases. To control for the specificity of the Helios/Ikaros protein interactions, the IkM1 (Ik-1 mutant) was also used in these assays. IkM1 encodes two point mutations in the C-terminal zinc fingers of Ikaros that disrupt the ability to dimerize. In contrast to Ik-1, this dimerization deficient form of Ikaros was unable to interact with either Helios isoform. Finally, cells were co-transfected with FLAG-Aiolos and either Hel-1 or Hel-2 to show that each Helios isoform can form heterodimers with Aiolos.. These studies show that the C-terminal zinc fingers in Helios, Ikaros and Aiolos are functionally conserved and mediate the stable interactions between these proteins which may be critical for hematopoiesis as well as lymphocyte differentiation and function.

Helios is part of a higher order nuclear structure that contains Ikaros and Aiolos

Our studies with Ikaros and Aiolos have shown that these proteins are part of a higher order structure in resting lymphocytes that undergoes dramatic changes upon activation. To determine whether Helios also participates in these nuclear macromolecular structures we examined its subcellular localization in primary lymphoid cells by confocal immunofluorescence microscopy. Primary thymocyte or splenocyte were obtained. Thymocyte were activated for 40 hours on plated precoated with 20 µg/ml CD3. Cells were harvested and washed in phosphate buffered saline (PBS). 1×10^5 cells were cytopun per slide and fixed 4% paraformaldehyde, 0.5% TWEEN in PBS at 4°C and then washed in PBS. Prior to antibody incubation, cells were blocked for 1 hour in 3%BSA, 1% goat serum, 1% donkey serum in PBS. Slides were then incubated with 1/50 dilution of primary affinity purified a Helios antibody in blocking buffer overnight at 4°C, followed by a 60 min. incubation at room temperature with 5ng/µl biotinylated goat α-rabbit IgG followed by a 60 min. incubation at room temperature with 5ng/µl biotinylated goat α rabbit IgG (Jackson labs). Each antibody incubation step was followed by 3 washes in PBS. A 45 min. incubation with 5ng/1µl avidin-FITC (Southern Biotechnology Associates) in 1% dialyzed FCS/3%BSA in PBS was done for detection. For double staining, an overnight

4°C incubation with affinity polyclonal Aiolos directly couple to the Alexa 568 flourophore (Molecular Probes) was done as the final step. For triple staining of Helios, Aiolos and the FLAG tagged IK-7 in cells from transgenic mice, were additionally incubated for 60 minutes at RT with 5ng/1µl of an anti-FLAG M5 monoclonal antibody (Kodak, washed
5 and then incubated for 60 minutes with a 5ng/µl Cy5 coupled goat anti-mouse antibody. Specific staining was visualized by confocal immunofluorescence microscopy .

Resting or activated thymocytes and splenocytes isolated from wild-type mice were prepared for these confocal studies. In contrast to Ikaros and Aiolos, bright staining for Helios was detected only in a small number of either resting or activated thymocytes
10 (approximately 1 in 25 cells). In these few cells, Helios was detected in a punctate pattern within the nucleus, similar to that previously described for Ikaros and Aiolos . Upon thymocyte activation, Helios was redistributed into ring like structures in the nucleus, as are Ikaros and Aiolos. Helios was also detected in a very small number of splenocytes. The cells are likely to be T or NK cells, as RT-PCR analysis indicated that Helios was not
15 expressed at significant levels in mature B cells, myeloid or erythroid cells.

To determine potential co-localization of these proteins in higher order structures, splenocytes were double stained for Aiolos and Helios. Although most cells in the spleen express Aiolos, a small subset of splenocytes express Helios as well. In most cases, there is complete overlap of these two proteins in a punctate pattern with the nucleus. However,
20 there are a few small spots where either Helios or Aiolos is detected alone. In addition, a few cells were observed that showed bright staining for Helios, but only faint staining for Aiolos. Cells stained for Ikaros and Helios showed a similar co-localization of the proteins.

To further investigate the nuclear localization of Helios with Ikaros and Aiolos, T cells from the spleen of mice expressing the FLAG-Ik-7 transgene were used. The FLAG-
25 epitope was utilized in triple staining studies to examine the localization of Ik-7 with the endogenous Helios and Aiolos proteins. In cells of young animals, these three proteins co-localize within nuclear structures, similar to that observed in wild type cells.

These studies establish the presence of all three family members in the same structures within the nucleus and demonstrate that Ikaros DN mutant proteins have the
30 potential to interfere with the activity of the endogenous Helios and Aiolos proteins by co-localization within the same macromolecular nuclear structures. As inferred from the

expression profiles of sorted cells, this immunofluorescence data also confirms the co-expression of different Ikaros family proteins in varying combinations within cells of distinct sub-populations in the thymus and spleen

5 **Helios can function as Transcriptional Activator**

Ikaros and Aiolos have been shown to function as positive transcriptional regulators upon ectopic expression in mammalian cells . The transcriptional activation domains of both proteins were identified using yeast one hybrid assays, and they were found to function similarly in mammalian cells. Helios protein exhibits conservation to the transcriptional
10 activation domain of Ikaros and Aiolos. Given the near identity in the DNA binding domain between Helios and Ikaros, we tested the ability of Helios to activate transcription from Ikaros binding sites. The expression of a reporter gene under the control of four high affinity Ikaros binding sites (IkBS2) was tested in the presence of Helios or Ikaros in NIH3T3 cells. Both proteins were shown to increase expression of the reporter gene over
15 background levels (Figure 3). A five fold increase was detected in the presence of Helios while a 7.8 fold increase was detected in the presence of Ikaros. This transcriptional activation mediated by Helios requires the Ikaros consensus binding sites. These results confirm the functional conservation of both the DNA binding and transcriptional activation domains.

20

Helios

The present invention identifies and characterizes Helios, a new member of the Ikaros gene family. The proteins encoded by all three genes in the Ikaros family share grossly similar properties mediated by conserved functional domains. All three bind to the
25 consensus DNA binding sites characterized for Ikaros and activate transcription from an adjacent promoter in co-transfection assays. Like Aiolos and Ikaros, Helios can dimerize with itself as well as other family members including a dominant negative isoform of Ikaros. Although the conservation of these domains emphasizes the similarity of these proteins, other regions differ between the proteins encoded by these genes and may confer
30 functional specificity among them. The fact that the regions that diverge between family

members are conserved in the orthologues of these genes in other species supports their functional significance.

The preferential expression of Helios in the earliest stages of the hemopoietic lineages suggests that gene may exert its predominant function in early progenitor cells.

- 5 The facts that a dominant negative Ikaros protein causes defects in the HSC and that Helios is the only identified target of this protein expressed at this stage of the lineage imply a crucial role for Helios in HSC development. The expression of Helios outside the hemopoietic system may indicate a role for the Ikaros gene family in progenitor development in other tissues as well.

10

Equivalents

Those skilled in the art will be able to recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention

- 15 and are covered by the following claims.

Claims:

1. A substantially pure nucleic acid comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, 3, or 5.

5 2. The nucleic acid of claim 1 comprising the nucleotide sequence of SEQ ID NO:1, 3 or 5.

 3. The nucleic acid of claim 1, wherein the nucleic acid hybridizes under high stringency conditions to a nucleic acid which encodes a Helios polypeptide, or a
10 complement thereof.

 4. The nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide that reacts with an antibody specific for a Helios polypeptide.

15 5. A substantially pure nucleic acid which encodes a fragment of a Helios polypeptide of at least 60 amino acids in length.

 6. A substantially pure polypeptide which is at least 60% homologous to an Helios polypeptide.
20

 7. The Helios polypeptide of claim 6 comprising the amino acid sequence of SEQ ID NO:2, 4, or 6.

 8. The pure preparation of claim 6, wherein the Helios polypeptide has the
25 following properties:

- (a) it can form a dimer with an Helios, Aiolos, or Ikaros polypeptide;
- (b) it is expressed in hematopoietic stem cells;
- (c) it has a molecular weight of approximately 64 kDa or 68 KDa;
- (d) it has at least one zinc finger domain; and
- 30 (e) it is a transcriptional activator of a lymphoid gene.

9. A fragment of the Helios polypeptide of claim 8 which is at least 50 amino acids in length.

5 10. A vector comprising the nucleic acid of any of claims 1-5.

11. A cell comprising the nucleic acid of any of claims 1-5 and 10. .

12. A purified preparation of an anti-Helios antibody.

10

13. A method for manufacture of an Helios peptide comprising culturing the cell of claim 11 in a medium to express the Helios polypeptide.

15 14. A method of making an Helios polypeptide, having at least one biological activity of a naturally occurring Helios polypeptide including altering the sequence, of one or more residues and testing the altered polypeptide for the desired activity.

20 15. A method for treating an animal for a disorder comprising administering a therapeutically-effective amount of an Helios polypeptide, a cell selected for the expression of a product of the Helios gene, or a nucleic acid encoding an Helios peptide to the animal.

25

16. A method for determining if a subject is at risk for a disorder related to mis-expression of the Helios gene comprising examining the subject for the expression or structure of the Helios gene, non-wildtype structure or expression being indicative of risk.

17. A transgenic animal having an Helios transgene.

Helios **MEYDAIDGYITCDNELSP**.....**EGEHANHAIBLTSSIPNSQNASPSMHTSTNSYLEMOSDEECDRQPLSREDHIRGHMGGSSLEEPLIES**
 Ikaros **EDVDDEGQDMSQVSGKESPPYSPTPD****EGDEPHVPEDLSTISGAQNSKSPRGNASVKTETOSDEENGAC**.....**EMNGEECAEDIRHLDASG**
 Aiolos **MEDIQPTVELKSTEEQPLPTESPDALNDYSLPKPDEIENVDSREAPNEDDAGEDSKYK**...**DEYSDRDENIMKPEPHGABESENPPYSYARE**

ZF 1 **ZF 2** **Ik-F** → **ZF 3**

Helios **SEVADHRYVD**...**EQEGGIRLPNGKLCBYCGVVCIGPNVLMYHKRSHTGERPFLCHQCGASFTQKGNLLRHKLHSGEKPFKCLFCSYACRR**
 Ikaros **EKNYSGSRDQSSALSGVGGIRLPNGKLCBYCGVVCIGPNVLMYHKRSHTGERPFLCHQCGASFTQKGNLLRHKLHSGEKPFKCHLCYACRN**
 Aiolos **YSBYESHKLENNVPY**...**DSRPTSGKLCBYCGVVCISFNVLMYHKRSHTGERPFLCHQCGASFTQKGNLLRHKLHSGEKPFKCHLCYACRN**

ZF 4

Helios **RDALTGHLRTHSYGKPHKCYICGRSYKOR****SLEEHKERCHNYLUVSHRAAGQVMSNVPPHEDCKEQEPIDNNISLYPFERPAVIEELTANQ**
 Ikaros **RDALTGHLRTHSYGKPHKCYICGRSYKORS****SLEEHKERCHNYLSHGLPQVCPV**...**KETNNE**...**MAEDLCKIGAESLYLDRLASHVA**
 Aiolos **RDALTGHLRTHSYGKPHKCYICGRSYKORS****SLEEHKERCHNYLQVPLQVDAASVEARHLK**.....**ANUSERALYLDRLASHVA**

TAD

Helios **KRKSSIPQKFLGK**...**CLSDHPTD**...**SANYEKE**...**SHMDQAINNAI****YLGAESLRPLVOTPPPS**...**SEVYPVISSM**...**OLKEPTSBGPPTS**
 Ikaros **KRKSSIPQKFLGK**...**CLSDHPTD**...**SANYEKE**...**SHMDQAINNAI****YLGAESLRPLVOTPPPS**...**SEVYPVISSM**...**OLKEPTSBGPPTS**
 Aiolos **KRKSSIPQKFLGK**...**CLSDHPTD**...**SANYEKE**...**SHMDQAINNAI****YLGAESLRPLVOTPPPS**...**SEVYPVISSM**...**OLKEPTSBGPPTS**

Helios **RIISDSHEHNDQPISTIRPKSLPQEREASPSNSCLDSTDSESSHDRQS**...**YOGEPALIPKPKQSPAYKEDVVALBATKAPKGS****SLDITTYKTF**
 Ikaros **...HNSAQDAVDHLLLSKAKSVSSEREASPSNSCLDSTDSESSHDRQS**...**GLIYLTAE**...**IPFARIGLA**...**LKEQORATVURAASENSQDAFRV**
 Aiolos **...GAPQEGEKRIELPEKILPSENGISPSNSAQDSTDSESSHDRQS**...**HLIQQSHVYLPQARNGPLKEVPRSPFLKPPPICLNDSTIKI**

ZF 5 **ZF 6**

Helios **EGEGEQLRAFKEHCRVLFLDHVMYTIHNGCHGIRDPLECMICGYSQDRIEFSSSHIVG6GHTFH**...
 Ikaros **STSGEQLKVVKEHCRVLFLDHVMYTIHNGCHGFRDPFECMCGYISQDRIEFSSSHIRGEHRYHLS**
 Aiolos **KEGEVMDVFC****HCIVLFLDHVMYTIHNGCHGFRDPFECMCGYISQDRIEFSSSHIRGEHRYHLS**

← **Ik-R**

FIG. 1

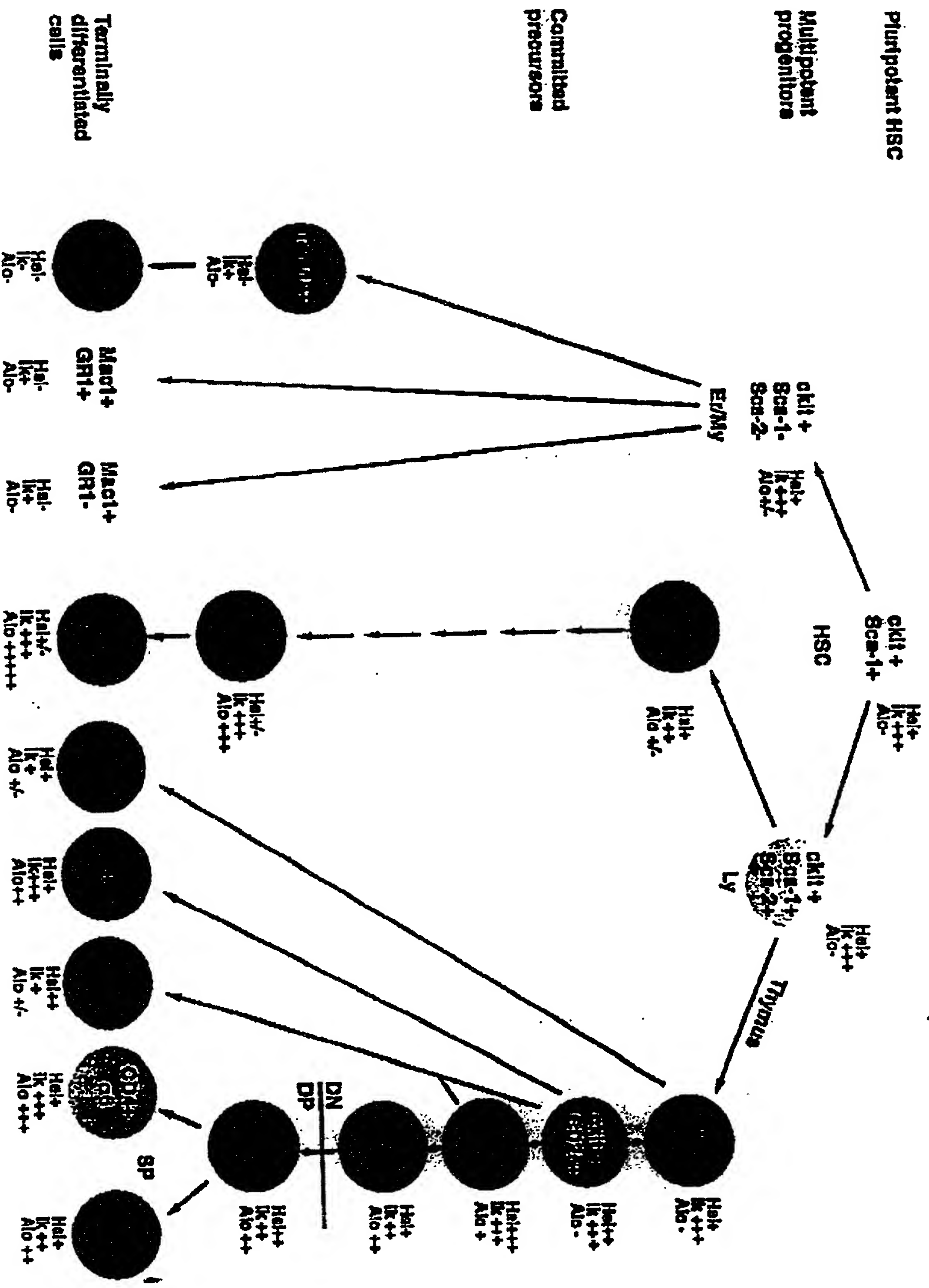


FIG. 2

ATGGAACAGACGCTATTGATGGCTATATAACATGTGACAATGAGCTTTCACCCGAAGGGGAACACGCCA
 TACCTTTGTCTGCGATAACTACCGATATATTGTACACTGTTACTCGAAAGTGGGCTTCCCCTTGTGCGGT
 M E T D A I D G Y I T C D N E L S P E G E H A
 ATATGGCCATTGACCTCACCTCAAGCAGGCCCAATGGACAGCAGCCTCGCCAAGTCACATGACAAGCAC
 TATACCGGTAAGTGGAGTGGAGTTCGTGCGGGTTACCTGTCGTGCGGAGCGGTTCAAGTGTACTGTTTCGTG
 N M A I D L T S S T P N G Q H A S P S H M T S T
 AAATTCTGTAAAGCTGGAAATGCAGAGTGATGAAGAGTGTGACAGGCAGCCCCCTGAGCCGTGAGGATGAG
 TTTAAGACATTTTCGACCTTTACGTCTCACTACTTCTCACACTGTCCGTGCGGGACTCGGCACTCCTACTC
 N S V K L E M Q S D E E C D R Q P L S R E D E
 ATCAGGGGCCACGATGAGGGGAGCAGCCTAGAAGAACCCTAATTGAGAGCAGCGAGGTGGCCGACAACA
 TAGTCCCCGGTGCTACTCCCCCTCGTCGGATCTTCTTGGGGATTAAGTCTCGTCGCTCCACCGGCTGTTGT
 I R G H D E G S S L E E P L I E S S E V A D N
 GGAAAGTCCAGGACCTTCAAGGCGAGGGAGGAATCCGGCTTCCGAATGGTAAACTGAAATGTGACGTCTG
 CCTTTCAGGTCTGGAAGTTCGCTCCCTCCTTAGGCCGAAGGCTTACCATTGACTTTACACTGCAGAC
 R K V Q D L Q G E G G I R L P N G K L K C D V C
 TGGCATGGTTTGCATTGGGCCCCAATGTGCTTATGGTACATAAAAGGAGTCACACTGGTGAGCGGCCCTTC
 ACCGTACCAAACGTAACCCGGGTACACGAATACCATGTATTTTCCTCAGTGTGACCACTCGCCGGGAAG
 G M V C I G P N V L M V H K R S H T G E R P F
 CACTGTAACCAGTGCGGAGCTTCTTTTACCCAGAAGGGCAACCTTCTGAGACAGATAAAGTTACACTCTG
 GTGACATTGGTCACGCCTCGAAGAAAATGGGTCTTCCCGTTGGAAGACTCTGTGTATTTCATGTGAGAC
 H C N Q C G A S F T Q K G N L L R H I K L H S
 GAGAGAAGCCCTTCAAATGTCCTTTCTGTAGCTATGCTTGTAGAAGAAGGGACGCTCTCACAGGACACCT
 CTCTCTTCGGGAAGTTTACAGGAAAGACATCGATACGAACATCTTCTTCCCTGCGAGAGTGTCTGTGGA
 G E K P F K C P F C S Y A C R R R D A L T G H L
 CAGGACCCATTCTGTGGGTAAACCTCACAAGTGTAAGTGTGAGGCGGAGCTACAAGCAGCGCACGTCA
 GTCCTGGGTAAAGACACCCATTTGGAGTGTTACATTGATGACACCGGCTTCGATGTTTCGTGCGGTGCAGT
 R T H S V G K P H K C N Y C G R S Y K Q R T S

FIG. 3
 (1 of 3)

CTGGAGGAACACAAGGAACGCTGTCACAACTATCTCCAGAATGTCAGCATGGAGGCTGCCGGGCAGGTCA 700
 GACCTCCTTGTGTTCTTGCGACAGTGTTGATAGAGGTCTTACAGTCGTACCTCCGACGGCCCGTCCAGT
 L E E H K E R C H N Y L Q N V S M E A A G Q V
 TGAGTCACCATGTACCGCCTATGGAAGATTGTAAGGAACAAGAGCCTATCATGGACAACAATATTTCTCT 770
 ACTCAGTGGTACATGGCGGATACCTTCTAACATTCTTGTCTCGGATAGTACCTGTTGTTATAAAGAGA
 M S H H V P P M E D C K E Q E P I M D N N I S L
 GGTGCCTTTTGAAGAGACCTGCTGTCATAGAGAAGCTCACGGCAAATATGGGAAAGCGCAAAAGCTCCACT 840
 CCACGGAAAACCTCTCTGGACGACAGTATCTCTTCGAGTGCCGTTTATACCCTTTTCGCGTTTTTCGAGGTGA
 V P F E R P A V I E K L T A N M G K R K S S T
 CCTCAGAAGTTTGTGGGGGAAAAGCTTATGCGATTTCAGCTACCCAGATATTCATTTTGATATGAACTTAA 910
 GGAGTCTTCAAACACCCCTTTTCGAATACGCTAAGTCGATGGGTCTATAAGTAAACTATACTTGAATT
 P Q K F V G E K L M R F S Y P D I H F D M N L
 CATATGAGAAGGAGGCTGAGCTGATGCAGTCTCATATGATGGACCAAGCCATCAACAATGCAATCACCTA 980
 GTATACTCTTCTCCGACTCGACTACGTCAGAGTATACTACCTGGTTCGGTAGTTGTTACGTTAGTGGAT
 T Y E K E A E L M Q S H M M D Q A I N N A I T Y
 CCTTGGAGCTGAGGCCCTTCACCCTCTGATGCAGCATGCACCAAGCACAATCGCTGAGGTGGCCCCAGTT 1050
 GGAACCTCGACTCCGGGAAGTGGGAGACTACGTCGTACGTGGTTCGTGTTAGCGACTCCACCGGGGTCAA
 L G A E A L H P L M Q H A P S T I A E V A P V
 ATAAGCTCAGCTTATTCTCAGGTCTATCATCCAAACAGGATAGAAAGACCCATTAGCAGGGAAACATCTG 1120
 TATTCGAGTCGAATAAGAGTCCAGATAGTAGGTTTGTCTATCTTTCTGGGTAATCGTCCCTTTGTAGAC
 I S S A Y S Q V Y H P N R I E R P I S R E T S
 ATAGTCACGAAAACAACATGGATGGCCCCATCTCTCTCATCAGACCAAGAGTCGACCCAGGAAAGAGA 1190
 TATCAGTGCTTTTGTGTACCTACCGGGGTAGAGAGAGTAGTCTGGTTTCTCAGCTGGGGTCCTTTCTCT
 D S H E N N M D G P I S L I R P K S R P Q E R E
 GGCCTCGCCCAGCAATAGCTGCCTCGATTCTACTGACTCAGAAAGTAGCCATGATGACCGCCAGTCCTAC 1260
 CCGGAGCGGGTCGTTATCGACGGAGCTAAGATGACTGAGTCTTTCATCGGTACTACTGGCGGTCAGGATG
 A S P S N S C L D S T D S E S S H D D R Q S Y

FIG. 3

(2 of 3)

CAAGGAAACCCTGCCTTAAATCCCAAGAGGAAACAAAGCCCAGCTTACATGAAGGAGGATGTCAAGGCTT 1330
 GTTCCTTTGGGACGGAATTTAGGGTTCTCCTTTGTTTCGGGTCGAATGTACTTCCTCCTACAGTTCCGAA
 Q G N P A L N P K R K Q S P A Y M K E D V K A
 TGGATGCTACCAAGGCCCCCAAGGGCTCTCTGAAGGACATCTATAAGGTTTTCAATGGAGAAGGAGAACA 1400
 ACCTACGATGGTTCCGGGGGTTCCTGAGAGACTTCCTGTAGATATTCCAAAAGTTACCTCTTCCTCTTGT
 L D A T K A P K G S L K D I Y K V F N G E G E Q
 GATAAGGGCCTTCAAGTGTGAGCACTGCCGAGTCCTTTTTCTAGACCATGTCATGTACACCATTACATG 1470
 CTATTCCTCGGAAGTTCACACTCGTGACGGCTCAGGAAAAAGATCTGGTACAGTACATGTGGTAAGTGTAC
 I R A F K C E H C R V L F L D H V M Y T I H M
 GGTGCCATGGCTACCGGGACCCACTGGAATGCAACATCTGTGGCTACAGAAGCCAGGACCGCTACGAAT 1540
 CCAACGGTACCGATGGCCCTGGGTGACCTTACGTTGTAGACACCGATGTCTTCGGTCCTGGCGATGCTTA
 G C H G Y R D P L E C N I C G Y R S Q D R Y E
 TTTCATCACACATTGTTGGGGGGCAGCACACATTCCACTAGGCGTTTGCATTCCAAGG 1598
 AAAGTAGTGTGTAACAACCCCCGTCGTGTGTAAGGTGATCCGCAAACGTAAGGTTCC
 F S S H I V G G Q H T F H . A F A F Q G

FIG. 3
 (3 of 3)

ATGGAAACAGACGCTATTGATGGCTATATAACATGTGACAATGAGCTTTCACCCGAAGGGGAACACGCCA
 TACCTTTGTCTGCGATAACTACCGATATATTGTACACTGTTACTCGAAAGTGGGCTTCCCCTTGTGCGGT
 M E T D A I D G Y I T C D N E L S P E G E H A 70
 ATATGGCCATTGACCTCACCTCAAGCACGCCCAATGGACAGCACGCCTCGCCAAGTCACATGACAAGCAC
 TATACCGGTAACCTGGAGTGGAGTTCGTGCGGGTTACCTGTCGTGCGGAGCGGTTTCAGTGTACTGTTCTGTG
 N M A I D L T S S T P N G Q H A S P S H M T S T 140
 AAATTCTGTAAAGCTGGAAATGCAGAGTGATGAAGAGTGTGACAGGCAGCCCCTGAGCCGTGAGGATGAG
 TTTAAGACATTTTCGACCTTTACGTCTCACTACTTCTCACACTGTCCGTCGGGGACTCGGCACTCCTACTC
 N S V K L E M Q S D E E C D R Q P L S R E D E 210
 ATCAGGGGCCACGATGAGGGGAGCAGCCTAGAAGAACCCTAATTGAGAGCAGCGAGGTGGCCGACAACA
 TAGTCCCCGGTGCTACTCCCTCGTCGGATCTTCTTGGGGATTAACCTCTCGTCGCTCCACCGGCTGTTGT
 I R G H D E G S S L E E P L I E S S E V A D N 280
 GGAAAGTCCAGGACCTTCAAGGCGAGGGAGGAATCCGGCTTCCGAATGGTGAGCGGCCCTTCCACTGTAA
 CCTTTCAGGTCCTGGAAGTTCGGCTCCCTCCTTAGGCCGAAGGCTTACCACTCGCCGGGAAGGTGACATT
 R K V Q D L Q G E G G I R L P N G E R P F H C N 350
 CCAGTGCGGAGCTTCTTTTACCCAGAAGGGCAACCTTCTGAGACACATAAAGTTACACTCTGGAGAGAAG
 GGTACGCCTCGAAGAAAATGGGTCTTCCCGTTGGAAGACTCTGTGTATTTCATGTGAGACCTCTCTTC
 Q C G A S F T Q K G N L L R H I K L H S G E K 420
 CCCTTCAAATGTCCTTTCTGTAGCTATGCTTGTAGAAGAAGGGACGCTCTCACAGGACACCTCAGGACCC
 GGGAAGTTTACAGGAAAGACATCGATACGAACATCTTCTTCCCTGCGAGAGTGTCTGTGGAGTCCTGGG
 P F K C P F C S Y A C R R R D A L T G H L R T 490
 ATTCTGTGGGTAAACCTCACAAGTGTAACCTACTGTGGCCGAAGCTACAAGCAGCGCACGTCCTGAGGGA
 TAAGACACCCATTTGGAGTGTTACATTGATGACACCGGCTTCGATGTTCTGTCGCGTGCAGTGACCTCCT
 H S V G K P H K C N Y C G R S Y K Q R T S L E E 560
 ACACAAGGAACGCTGTCACAACCTATCTCCAGAATGTCAGCATGGAGGCTGCCGGGCAGGTCATGAGTCAC
 TGTGTTCTTTCGACAGTGTTGATAGAGGTCTTACAGTCGTACCTCCGACGGCCCGTCCAGTACTCAGTG
 H K E R C H N Y L Q N V S M E A A G Q V M S H 630

FIG. 4
 (1 of 3)

CATGTACCGCCTATGGAAGATTGTAAGGAACAAGAGCCTATCATGGACAACAATATTTCTCTGGTGCCTT
 GTACATGGCGGATACCTTCTAACATTCCTTGTTCTCGGATAGTACCTGTTGTTATAAAGAGACCACGGAA 700
 H V P P M E D C K E Q E P I M D N N I S L V P
 TTGAGAGACCTGCTGTCATAGAGAAGCTCACGGCAAATATGGGAAAGCGCAAAAGCTCCACTCCTCAGAA 770
 AACTCTCTGGACGACAGTATCTCTTCGAGTGCCGTTTATACCCTTTTCGCGTTTTCGAGGTGAGGAGTCTT
 F E R P A V I E K L T A N M G K R K S S T P Q K
 GTTTGTGGGGGAAAAGCTTATGCGATTCAGCTACCCAGATATTCATTTTGATATGAACTTAACATATGAG 840
 CAAACACCCCTTTTCGAATACGCTAAGTCGATGGGTCTATAAGTAAACTATACTTGAATTGTATACTC
 F V G E K L M R F S Y P D I H F D M N L T Y E
 AAGGAGGCTGAGCTGATGCAGTCTCATATGATGGACCAAGCCATCAACAATGCAATCACCTACCTTGGAG 910
 TTCCTCCGACTCGACTACGTCAGAGTATACTACCTGGTTCGGTAGTTGTTACGTTAGTGGATGGAACCTC
 K E A E L M Q S H M M D Q A I N N A I T Y L G
 CTGAGGCCCTTCACCCTCTGATGCAGCATGCACCAAGCACAATCGCTGAGGTGGCCCCAGTTATAAGCTC 980
 GACTCCGGGAAGTGGGAGACTACGTCGTACGTGGTTCGTGTTAGCGACTCCACCGGGGTCAATATTCGAG
 A E A L H P L M Q H A P S T I A E V A P V I S S
 AGCTTATTCTCAGGTCTATCATCCAAACAGGATAGAAAGACCCATTAGCAGGGAAACATCTGATAGTCAC 1050
 TCGAATAAGAGTCCAGATAGTAGGTTTGTCTATCTTTCTGGGTAATCGTCCCTTTGTAGACTATCAGTG
 A Y S Q V Y H P N R I E R P I S R E T S D S H
 GAAAACAACATGGATGGCCCCATCTCTCTCATCAGACCAAGAGTCGACCCCAGGAAAGAGAGGCCTCGC 1120
 CTTTTGTTGTACCTACCGGGGTAGAGAGAGTAGTCTGGTTTCTCAGCTGGGGTCCTTTCTCTCCGGAGCG
 E N N M D G P I S L I R P K S R P Q E R E A S
 CCAGCAATAGCTGCCTCGATTCTACTGACTCAGAAAGTAGCCATGATGACCGCCAGTCCTACCAAGGAAA 1190
 GGTCGTTATCGACGGAGCTAAGATGACTGAGTCTTTCATCGGTACTACTGGCGGTCAGGATGGTTCCTTT
 P S N S C L D S T D S E S S H D D R Q S Y Q G N
 CCCTGCCTTAAATCCCAAGAGGAAACAAAGCCCAGCTTACATGAAGGAGGATGTCAAGGCTTTGGATGCT 1260
 GGGACGGAATTTAGGGTTCTCCTTTGTTTCGGGTCGAATGTACTTCCTCCTACAGTTCGAAACCTACGA
 P A L N P K R K Q S P A Y M K E D V K A L D A

FIG. 4
 (2 of 3)

ACCAAGGCCCCCAAGGGCTCTCTGAAGGACATCTATAAGGTTTTCAATGGAGAAGGAGAACAGATAAGGG 1330
TGGTTCCGGGGGTTCCCGAGAGACTTCCTGTAGATATTCCAAAAGTTACCTCTTCCTCTTGTCTATTCCC
T K A P K G S L K D I Y K V F N G E G E Q I R
CCTTCAAGTGTGAGCACTGCCGAGTCCTTTTTCTAGACCATGTCATGTACACCATTACATGGGTTGCCA 1400
GGAAGTTCACACTCGTGACGGCTCAGGAAAAAGATCTGGTACAGTACATGTGGTAAGTGTACCCAACGGT
A F K C E H C R V L F L D H V M Y T I H M G C H
TGGCTACCGGGACCCACTGGAATGCAACATCTGTGGCTACAGAAGCCAGGACCGCTACGAATTTTCATCA 1470
ACCGATGGCCCTGGGTGACCTTACGTTGTAGACACCGATGTCTTCGGTCCTGGCGATGCTTAAAAGTAGT
G Y R D P L E C N I C G Y R S Q D R Y E F S S
CACATTGTTGGGGGGCAGCACACATTCCACTAGGCGTTTGCATTCCAAGG 1520
GTGTAACAACCCCCCGTCGTGTGTAAGGTGATCCGCAAACGTAAGGTTCC
H I V G G Q H T F H . A F A F Q G

FIG. 4
(3 of 3)

| | |
|---|---|
| 1/1 | 31/11 |
| GCC CGG GCA GGT CGC ATT GCT ATA GCA CTG | ACT GAC CTC TCT CTC TCT CTT TTT TTT CCT |
| A R A G R I A I A L | T D L S L S L F F P |
| 61/21 | 91/31 |
| CTT TCC TGA AAC CCG ACA TTG TCA CCT CCT | CTT TGA GGG TTA GAA GAA GCT GAG ATC TCC |
| L S * N P T L S P P | L * G L E E A E I S |
| 121/41 | 151/51 |
| CGA CAG AGC TGG AAA TGG TGA TGA ATC TTT | TTT AAT CAA AGG ACA ATT TCT TTT CAT TGC |
| R Q S W K W * * I F | F N Q R T I S F H C |
| 181/61 | 211/71 |
| ACT TTG ACT ATG GAA ACA GAG GCT ATT GAT | GGC TAT ATA ACG TGT GAC AAT GAG CTT TCA |
| T L T M E T E A I D | G Y I T C D N E L S |
| 241/81 | 271/91 |
| CCC GAA AGG GAG CAC TCC AAT ATG GCA ATT | GAC CTC ACC TCA AGC ACA CCC AAT GGA CAG |
| P E R E H S N M A I | D L T S S T P N G Q |
| 301/101 | 331/111 |
| CAT GCC TCA CCA AGT CAC ATG ACA AGC ACA | GAT TCA GTA AAG CTA GAA ATG CAG AGT GAT |
| H A S P S H M T S T | D S V K L E M Q S D |
| 361/121 | 391/131 |
| GAA GAG TGT GAC AGG AAA CCC CTG AGC CGT | GAA GAT GAG ATC AGG GGC CAT GAT GAG GGT |
| E E C D R K P L S R | E D E I R G H D E G |
| 421/141 | 451/151 |
| AGC AGC CTA GAA GAA CCC CTA ATT GAG AGC | AGC GAG GTG GCT GAC AAC AGG GAA GTC CAG |
| S S L E E P L I E S | S E V A D N R E V Q |
| 481/161 | 511/171 |
| GAG CTT CAA GGC GAG GGA GGA ATC CGG CTT | CCG AAT GGT AAA CTG AAA TGT GAC GTC TGT |
| E L Q G E G G I R L | P N G K L K C D V C |
| 541/181 | 571/191 |
| GGC ATG GTT TGC ATT GGG CCC AAT GTG CTT | ATG GTA CAT AAA AGG AGT CAC ACT GGT GAA |
| G M V C I G P N V L | M V H K R S H T G E |
| 601/201 | 631/211 |
| CGC CCC TTC CAC TGT AAC CAG TGT GGA GCT | TCT TTT ACT CAG AAG GGC AAC CTT CTG AGA |
| R P F H C N Q C G A | S F T Q K G N L L R |
| 661/221 | 691/231 |
| CAC ATA AAG TTA CAC TCT GGA GAG AAG CCG | TTC AAA TGT CCT TTC TGT AGT CAC GCC TGT |
| H I K L H S G E K P | F K C P F C S H A C |
| 721/241 | 751/251 |
| TGA AGA AGG GAC GCC CTC ACA GGA TAC CTC | AGG ACC CAT TCT GTG GGT AAA CCT CAC AAG |
| R R R D A L T G Y L | R T H S V G K P H K |
| 781/261 | 811/271 |
| TGC AAC TAC TGT GGA CGA AGC TAC AAG CAG | CGC AGT TCA CTG GAG GAG CAC AAG GAA CGC |
| C N Y C G R S Y K Q | R S S L E E H K E R |
| 841/281 | 871/291 |
| TGC CAC AAC TAT CTC CAG AAT GTC AGC ATG | GAG GCT GCT GGG CAG GTC ATG AGT CAC CAT |
| C H N Y L Q N V S M | E A A G Q V M S H H |
| 901/301 | 931/311 |
| GTA CCT CCT ATG GAA GAT TGT AAG GAA CAA | GAG CCT ATT ATG GAC AAC AAT ATT TCT CTG |
| V P P M E D C K E Q | E P I M D N N I S L |
| 961/321 | 991/331 |
| GTG CCT TTT GAG AGA CCT GCT GTC ATA GAG | AAG CTC ACG GGG AAT ATG GGA AAA CGT AAA |
| V P F E R P A V I E | K L T G N M G K R K |
| 1021/341 | 1051/351 |
| AGC TCC ACT CCA CAA AAG TTT GTG GGG GAA | AAG CTC ATG CGA TTC AGC TAC CCA GAT ATT |
| S S T P Q K F V G E | K L M R F S Y P D I |
| 1081/361 | 1111/371 |
| CAC TTT GAT ATG AAC TTA ACA TAT GAG AAG | GAG GCT GAG CTG ATG CAG TCT CAT ATG ATG |
| H F D M N L T Y E K | E A E L M Q S H M M |
| 1141/381 | 1171/391 |
| GAC CAA GCC ATC AAC AAT GCA ATC ACC TAC | CTT GGA GCT GAG GCC CTT CAC CCT CTG ATG |
| D Q A I N N A I T Y | L G A E A L H P L M |
| 1201/401 | 1231/411 |
| CAG CAC CCG CCA AGC ACA ATC GCT GAA GTG | GCC CCA GTT ATA AGC TCA GCT TAT TCT CAG |
| Q H P P S T I A E V | A P V I S S A Y S Q |

FIG. 5
(1 of 2)

| | |
|---|---|
| 1261/421 | 1291/431 |
| GTC TAT CAT CCA AAT AGG ATA GAA AGA CCC | ATT AGC AGG GAA ACT GCT GAT AGT CAT GAA |
| V Y H P N R I E R P | I S R E T A D S H E |
| 1321/441 | 1351/451 |
| AAC AAC ATG GAT GGC CCC ATC TCT CTC ATC | AGA CCA AAG AGT CGA CCC CAG GAA AGA GAG |
| N N M D G P I S L I | R P K S R P Q E R E |
| 1381/461 | 1411/471 |
| GCC TCT CCC AGC AAT AGC TGC CTG GAT TCC | ACT GAC TCA GAA AGC AGC CAT GAT GAC CAC |
| A S P S N S C L D S | T D S E S S H D D H |
| 1441/481 | 1471/491 |
| CAG TCC TAC CAA GGA CAC CCT GCC TTA AAT | CCC AAG AGG AAA CAA AGC CCA GCT TAC ATG |
| Q S Y Q G H P A L N | P K R K Q S P A Y M |
| 1501/501 | 1531/511 |
| AAG GAG GAT GTC AAA GCT TTG GAT ACT ACC | AAG GCT CCT AAG GGC TCT CTG AAG GAC ATC |
| K E D V K A L D T T | K A P K G S L K D I |
| 1561/521 | 1591/531 |
| TAC AAG GTC TTC AAT GGG GAA GGA GAA CAG | ATT AGG GCC TTC AAG TGT GAG CAC TGC CGA |
| Y K V F N G E G E Q | I R A F K C E H C R |
| 1621/541 | 1651/551 |
| GTC CTT TTC CTA GAC CAT GTC ATG TAC ACC | ATT CAC ATG GGT TGC CAT GGC TAC CGG GAC |
| V L F L D H V M Y T | I H M G C H G Y R D |
| 1681/561 | 1711/571 |
| CCA CTG GAA TGT AAC ATC TGT GGC TAC AGA | AGC CAG GAC CGT TAT GAG TTT TCA TCA CAC |
| P L E C N I C G Y R | S Q D R Y E F S S H |
| 1741/581 | 1771/591 |
| ATT GTT CGA GGG GAG CAC ACA TTC CAC TAG | GCC TTT TCA TTC CAA AGG GGA CCC TAT GAA |
| I V R G E H T F H * | A F S F Q R G P Y E |
| 1801/601 | 1831/611 |
| GTA AAG ACT GCA CAT GAA GAA ATA CTG CAC | TTA CAA TCC CAC CTT TCC TCA AAT GTT GTA |
| V K T A H E E I L H | L Q S H L S S N V V |
| 1861/621 | 1891/631 |
| CCT TTT ATT TTT TTA ATA TAA TAC TGG TGA | TAA TCT TAT TTT GTG GAG CAG TGT CAT TTG |
| P F I F L I * Y W * * | S Y F V E Q C H L |
| 1921/641 | |
| CTC TGC T | |
| L C | |

FIG. 5
(2 of 2)

1 ATGGAAACAGACGCTATTGATGGCTATATAACATGTGACAATGAGCTTTC 50
190 ATGGAAACAGAGGCTATTGATGGCTATATAACGTGTGACAATGAGCTTTC 239
51 ACCCGAAGGGGAACACGCCAATATGGCCATTGACCTCACCTCAAGCACGC 100
240 ACCCGAAGGGGAGCACTCCAATATGGCAATTGACCTCACCTCAAGCACAC 289
101 CCAATGGACAGCAcGCCTCGCCAAGTCACATGACAAGCACAAATTCTGTA 150
290 CCAATGGACAGCATGCCTCACCAAGTCACATGACAAGCACAGATTTCAGTA 339
151 AAGCTGGAAATGCAGAGTGATGAAGAGTGTGACAGGCAGCCCCTGAGCCG 200
340 AAGCTAGAAATGCAGAGTGATGAAGAGTGTGACAGGAAACCCCTGAGCCG 389
201 TGAGGATGAGATCAGGGGCCACGATGAGGGGAGCAGCCTAGAAGAcCCC 250
390 TGAAGATGAGATCAGGGGCCATGATGAGGGTAGCAGCCTAGAAGAACCCC 439
251 TAATTGAGAGCAGCGAGGTGGCCGACAACAGGAAAGTCCAGGACCTTCAA 300
440 TAATTGAGAGCAGCGAGGTGGCTGACAACAGGGAAGTCCAGGAGCTTCAA 489
301 GGCGAGGGAGGAATCCGGCTTCCGAATGGTAAACTGAAATGTGACGTCTG 350
490 GGCGAGGGAGGAATCCGGCTTCCGAATGGTAAACTGAAATGTGACGTCTG 539
351 TGGCATGGTTTGCATTGGGCCCCAATGTGCTTATGGTACATAAAAGGAGTC 400
540 TGGCATGGTTTGCATTGGGCCCCAATGTGCTTATGGTACATAAAAGGAGTC 589
401 ACACTGGTGAGCGGCCCTTCCACTGTAACCAGTGCGGAGCTTCTTTTACC 450
590 ACACTGGTGAACGCCCTTCCACTGTAACCAGTGTGGAGCTTCTTTTACT 639
451 CAGAAGGGCAACCTTCTGAGACACATAAAGTTACACTCTGGAGAGAAGCC 500
640 CAGAAGGGCAACCTTCTGAGACACATAAAGTTACACTCTGGAGAGAAGCC 689
501 CTTCAAATGTCCTTTCTGTAGCTATGCTTGTAAGAAGGGACGCTCTCA 550
690 GTTCAAATGTCCTTTCTGTAGTCACGCCTGTAGAAGAAGGGACGCCCTCA 739
551 CAGGACACCTCAGGACCCATTCTGTGGGTAAACCTCACAAGTGTAACACTAC 600
740 CAGGATACCTCAGGACCCATTCTGTGGGTAAACCTCACAAGTGCAACTAC 789
601 TGTGGCCGAAGCTACAAGCAGCGCACGTCACTGGAGGAACACAAGGAACG 650
790 TGTGGACGAAGCTACAAGCAGCGCAGTTCCTGGAGGAGCACAAAGGAACG 839
651 CTGTCACAACCTATCTCCAGAATGTCAGCATGGAGGCTGCCGGGCAGGTCA 700
840 CTGCCACAACCTATCTCCAGAATGTCAGCATGGAGGCTGCTGGGCAGGTCA 889
701 TGAGTCACCATGTACCGCCTATGGAAGATTGTAAGGAACAAGAGCCTATC 750
890 TGAGTCACCATGTACCTCCTATGGAAGATTGTAAGGAACAAGAGCCTATT 939
751 ATGGACAACAATATTTCTCTGGTGCCTTTTGAGAGACCTGCTGTCATAGA 800

FIG. 6
(1 of 3)

940 ATGGACAACAATATTTCTCTGGTGCCTTTTGAGAGACCTGCTGTCATAGA 989
 801 GAAGCTCACGGCAAATATGGGAAAGCGCAAAGCTCCACTCCTCAGAAGT 850
 |||||
 990 GAAGCTCACGGGGAATATGGGAAAACGTAAAAGCTCCACTCCACAAAAGT 1039
 851 TTGTGGGGGAAAAGCTTATGCGATTTCAGCTACCCAGATATTCATTTTGAT 900
 |||||
 1040 TTGTGGGGGAAAAGCTCATGCGATTTCAGCTACCCAGATATTCACTTTGAT 1089
 901 ATGAACTTAACATATGAGAAGGAGGCTGAGCTGATGCAGTCTCATATGAT 950
 |||||
 1090 ATGAACTTAACATATGAGAAGGAGGCTGAGCTGATGCAGTCTCATATGAT 1139
 951 GGACCAAGCCATCAACAATGCAATCACCTACCTTGGAGCTGAGGCCCTTC 1000
 |||||
 1140 GGACCAAGCCATCAACAATGCAATCACCTACCTTGGAGCTGAGGCCCTTC 1189
 1001 ACCCTCTGATGCAGCATGCACCAAGCACAATCGCTGAGGTGGCCCCAGTT 1050
 |||||
 1190 ACCCTCTGATGCAGCACCCGCCAAGCACAATCGCTGAAGTGGCCCCAGTT 1239
 1051 ATAAGCTCAGCTTATTCTCAGGTCTATCATCCAAACAGGATAGAAAGACC 1100
 |||||
 1240 ATAAGCTCAGCTTATTCTCAGGTCTATCATCCAAATAGGATAGAAAGACC 1289
 1101 CATTAGCAGGGAAACATCTGATAGTCACGAAAACAACATGGATGGCCCCA 1150
 |||||
 1290 CATTAGCAGGGAAACTGCTGATAGTCATGAAAACAACATGGATGGCCCCA 1339
 1151 TCTCTCTCATCAGACCAAAGAGTCGACCCCAAGGAAAGAGAGGCCTCGCCC 1200
 |||||
 1340 TCTCTCTCATCAGACCAAAGAGTCGACCCCAAGGAAAGAGAGGCCTCTCCC 1389
 1201 AGCAATAGCTGCCTCGATTCTACTGACTCAGAAAGTAGCCATGATGACCG 1250
 |||||
 1390 AGCAATAGCTGCCTGGATTCCACTGACTCAGAAAGCAGCCATGATGACCA 1439
 1251 CCAGTCCTACCAAGGAAACCCTGCCTTAAATCCCAAGAGGAAACAAAGCC 1300
 |||||
 1440 CCAGTCCTACCAAGGACACCCTGCCTTAAATCCCAAGAGGAAACAAAGCC 1489
 1301 CAGCTTACATGAAGGAGGATGTCAAGGCTTTGGATGCTACCAAGGCCCCC 1350
 |||||
 1490 CAGCTTACATGAAGGAGGATGTCAAAGCTTTGGATACTACCAAGGCTCCT 1539
 1351 AAGGGCTCTCTGAAGGACATCTATAAGGTTTTCAATGGAGAAGGAGAACA 1400
 |||||
 1540 AAGGGCTCTCTGAAGGACATCTACAAGTCTTCAATGGGAAGGAGAACA 1589
 1401 GATAAGGGCCTTCAAGTGTGAGCACTGCCGAGTCCTTTTTCTAGACCATG 1450
 |||||
 1590 GATTAGGGCCTTCAAGTGTGAGCACTGCCGAGTCCTTTTCCTAGACCATG 1639
 1451 TCATGTACACCATTCACATGGGTTGCCATGGCTACCGGGACCCACTGGAA 1500
 |||||
 1640 TCATGTACACCATTCACATGGGTTGCCATGGCTACCGGGACCCACTGGAA 1689
 1501 TGCAACATCTGTGGCTACAGAAGCCAGGACCGCTACGAATTTTCATCACA 1550
 |||||
 1690 TGTAACATCTGTGGCTACAGAAGCCAGGACCGTTATGAGTTTTTCATCACA 1739

FIG. 6
 (2 of 3)

1551 CATTGTTGGGGGGCAGCACACATTCCACTAGGCGTTTGCATTCCAAGG 1598
||||||| | ||| ||||||||||||||||||| ||| ||||||| |
1740 CATTGTTCGAGGGGAGCACACATTCCACTAGGCCTTTTCATTCCAAAG 1787

FIG. 6
(3 of 3)

1 METEAIDGYITCDNELSPEREHSNMAIDLTSSSTPNGQHASP SHMTSTD SV 50
|||||
1 METEAIDGYITCDNELSPEREHSNMAIDLTSSSTPNGQHASP SHMTSTD SV 50

51 KLEMQSDEECDRKPLSREDEIRGHDEGSSLEEPLIESSEVADNREVQELQ 100
|||||
51 KLEMQSDEECDRKPLSREDEIRGHDEGSSLEEPLIESSEVADNREVQELQ 100

101 GEGGIRLPNGKCLKDVCGMVCIGPNVLMVHKRSHTGERPFFHCNQC GASFT 150
|||||
101 GEGGIRLPNGKCLKDVCGMVCIGPNVLMVHKRSHTGERPFFHCNQC GASFT 150

151 QKGNLLRHIKLSGEKPFKCPFCSHACRRRDALTGYLRTHSVGKPHKCNY 200
|||||
151 QKGNLLRHIKLSGEKPFKCPFCSHACRRRDALTGYLRTHSVGKPHKCNY 200

201 CGRSYKQRSSLEE HKERCHNYLQNVSM EAAGQVMSHHVPPMEDCKEQEPI 250
|||||
201 CGRSYKQRSSLEE HKERCHNYLQNVSM EAAGQVMSHHVPPMEDCKEQEPI 250

251 MDNNISLVPFERPAVIEKLTGNMGKRKSSTPQKFVGEKLMRFSYPDIHFD 300
|||||
251 MDNNISLVPFERPAVIEKLTGNMGKRKSSTPQKFVGEKLMRFSYPDIHFD 300

301 MNLTYEKEAELMQSHMMDQAINNAITYLGAEALHPLMQHPPSTIAEVAPV 350
|||||
301 MNLTYEKEAELMQSHMMDQAINNAITYLGAEALHPLMQHPPSTIAEVAPV 350

351 ISSAYSQVYHPNRIERPISRETADSHENNMDGPISLIRPKSRPQEREASP 400
|||||
351 ISSAYSQVYHPNRIERPISRETADSHENNMDGPISLIRPKSRPQEREASP 400

401 SNSCLDSTDSESSHDDHQSYQGH PALNPKRKQSPAYMKEDVKALD TTKAP 450
|||||
401 SNSCLDSTDSESSHDDHQSYQGH PALNPKRKQSPAYMKEDVKALD TTKAP 450

451 KGSLKDIYKVFNGEGEQIRAFKCEHCRVLF LDHVMYTIHMGCHGYRDPLE 500
|||||
451 KGSLKDIYKVFNGEGEQIRAFKCEHCRVLF LDHVMYTIHMGCHGYRDPLE 500

501 CNICGYRSQDRYEFSSHIVRGEHTFH 526
|||||
501 CNICGYRSQDRYEFSSHIVRGEHTFH 526

FIG. 7

SEQUENCE LISTING

<110> Georgopoulos, Katia
Mogan, Bruce A.
Kelly, Clair

<120> THE HELIOS GENE

<130> 10287/043001

<150> US 60/076,325

<151> 1998-02-27

<160> 17

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 1598

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(1578)

<400> 1

| | |
|---|-----|
| atg gaa aca gac gct att gat ggc tat ata aca tgt gac aat gag ctt | 48 |
| Met Glu Thr Asp Ala Ile Asp Gly Tyr Ile Thr Cys Asp Asn Glu Leu | |
| 1 5 10 15 | |
| tca ccc gaa ggg gaa cac gcc aat atg gcc att gac ctc acc tca agc | 96 |
| Ser Pro Glu Gly Glu His Ala Asn Met Ala Ile Asp Leu Thr Ser Ser | |
| 20 25 30 | |
| acg ccc aat gga cag cac gcc tcg cca agt cac atg aca agc aca aat | 144 |
| Thr Pro Asn Gly Gln His Ala Ser Pro Ser His Met Thr Ser Thr Asn | |
| 35 40 45 | |
| tct gta aag ctg gaa atg cag agt gat gaa gag tgt gac agg cag ccc | 192 |
| Ser Val Lys Leu Glu Met Gln Ser Asp Glu Glu Cys Asp Arg Gln Pro | |
| 50 55 60 | |
| ctg agc cgt gag gat gag atc agg ggc cac gat gag ggg agc agc cta | 240 |
| Leu Ser Arg Glu Asp Glu Ile Arg Gly His Asp Glu Gly Ser Ser Leu | |
| 65 70 75 80 | |
| gaa gaa ccc cta att gag agc agc gag gtg gcc gac aac agg aaa gtc | 288 |
| Glu Glu Pro Leu Ile Glu Ser Ser Glu Val Ala Asp Asn Arg Lys Val | |
| 85 90 95 | |
| cag gac ctt caa ggc gag gga gga atc cgg ctt ccg aat ggt aaa ctg | 336 |
| Gln Asp Leu Gln Gly Glu Gly Gly Ile Arg Leu Pro Asn Gly Lys Leu | |
| 100 105 110 | |
| aaa tgt gac gtc tgt ggc atg gtt tgc att ggg ccc aat gtg ctt atg | 384 |
| Lys Cys Asp Val Cys Gly Met Val Cys Ile Gly Pro Asn Val Leu Met | |
| 115 120 125 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| gta | cat | aaa | agg | agt | cac | act | ggt | gag | cgg | ccc | ttc | cac | tgt | aac | cag | 432 |
| Val | His | Lys | Arg | Ser | His | Thr | Gly | Glu | Arg | Pro | Phe | His | Cys | Asn | Gln | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| tgc | gga | gct | tct | ttt | acc | cag | aag | ggc | aac | ctt | ctg | aga | cac | ata | aag | 480 |
| Cys | Gly | Ala | Ser | Phe | Thr | Gln | Lys | Gly | Asn | Leu | Leu | Arg | His | Ile | Lys | |
| | 145 | | | | 150 | | | | | 155 | | | | | 160 | |
| tta | cac | tct | gga | gag | aag | ccc | ttc | aaa | tgt | cct | ttc | tgt | agc | tat | gct | 528 |
| Leu | His | Ser | Gly | Glu | Lys | Pro | Phe | Lys | Cys | Pro | Phe | Cys | Ser | Tyr | Ala | |
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| tgt | aga | aga | agg | gac | gct | ctc | aca | gga | cac | ctc | agg | acc | cat | tct | gtg | 576 |
| Cys | Arg | Arg | Arg | Asp | Ala | Leu | Thr | Gly | His | Leu | Arg | Thr | His | Ser | Val | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| ggt | aaa | cct | cac | aag | tgt | aac | tac | tgt | ggc | cga | agc | tac | aag | cag | cgc | 624 |
| Gly | Lys | Pro | His | Lys | Cys | Asn | Tyr | Cys | Gly | Arg | Ser | Tyr | Lys | Gln | Arg | |
| | | 195 | | | | 200 | | | | | | 205 | | | | |
| acg | tca | ctg | gag | gaa | cac | aag | gaa | cgc | tgt | cac | aac | tat | ctc | cag | aat | 672 |
| Thr | Ser | Leu | Glu | Glu | His | Lys | Glu | Arg | Cys | His | Asn | Tyr | Leu | Gln | Asn | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| gtc | agc | atg | gag | gct | gcc | ggg | cag | gtc | atg | agt | cac | cat | gta | ccg | cct | 720 |
| Val | Ser | Met | Glu | Ala | Ala | Gly | Gln | Val | Met | Ser | His | His | Val | Pro | Pro | |
| | 225 | | | | 230 | | | | | 235 | | | | | 240 | |
| atg | gaa | gat | tgt | aag | gaa | caa | gag | cct | atc | atg | gac | aac | aat | att | tct | 768 |
| Met | Glu | Asp | Cys | Lys | Glu | Gln | Glu | Pro | Ile | Met | Asp | Asn | Asn | Ile | Ser | |
| | | | | 245 | | | | | 250 | | | | | 255 | | |
| ctg | gtg | cct | ttt | gag | aga | cct | gct | gtc | ata | gag | aag | ctc | acg | gca | aat | 816 |
| Leu | Val | Pro | Phe | Glu | Arg | Pro | Ala | Val | Ile | Glu | Lys | Leu | Thr | Ala | Asn | |
| | | | 260 | | | | | 265 | | | | | 270 | | | |
| atg | gga | aag | cgc | aaa | agc | tcc | act | cct | cag | aag | ttt | gtg | ggg | gaa | aag | 864 |
| Met | Gly | Lys | Arg | Lys | Ser | Ser | Thr | Pro | Gln | Lys | Phe | Val | Gly | Glu | Lys | |
| | | 275 | | | | 280 | | | | | | 285 | | | | |
| ctt | atg | cga | ttc | agc | tac | cca | gat | att | cat | ttt | gat | atg | aac | tta | aca | 912 |
| Leu | Met | Arg | Phe | Ser | Tyr | Pro | Asp | Ile | His | Phe | Asp | Met | Asn | Leu | Thr | |
| | 290 | | | | | 295 | | | | | 300 | | | | | |
| tat | gag | aag | gag | gct | gag | ctg | atg | cag | tct | cat | atg | atg | gac | caa | gcc | 960 |
| Tyr | Glu | Lys | Glu | Ala | Glu | Leu | Met | Gln | Ser | His | Met | Met | Asp | Gln | Ala | |
| | 305 | | | | 310 | | | | | 315 | | | | | 320 | |
| atc | aac | aat | gca | atc | acc | tac | ctt | gga | gct | gag | gcc | ctt | cac | cct | ctg | 1008 |
| Ile | Asn | Asn | Ala | Ile | Thr | Tyr | Leu | Gly | Ala | Glu | Ala | Leu | His | Pro | Leu | |
| | | | | 325 | | | | | 330 | | | | | 335 | | |
| atg | cag | cat | gca | cca | agc | aca | atc | gct | gag | gtg | gcc | cca | gtt | ata | agc | 1056 |
| Met | Gln | His | Ala | Pro | Ser | Thr | Ile | Ala | Glu | Val | Ala | Pro | Val | Ile | Ser | |
| | | | 340 | | | | | 345 | | | | | 350 | | | |
| tca | gct | tat | tct | cag | gtc | tat | cat | cca | aac | agg | ata | gaa | aga | ccc | att | 1104 |
| Ser | Ala | Tyr | Ser | Gln | Val | Tyr | His | Pro | Asn | Arg | Ile | Glu | Arg | Pro | Ile | |
| | | 355 | | | | | 360 | | | | | 365 | | | | |

| | | | | | | | | | | | | | | | | |
|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| agc Ser 370 | agg Arg | gaa Glu | aca Thr | tct Ser | gat Asp | agt Ser 375 | cac His | gaa Glu | aac Asn | aac Asn | atg Met 380 | gat Asp | ggc Gly | ccc Pro | atc Ile | 1152 |
| tct Ser 385 | ctc Leu | atc Ile | aga Arg | cca Pro | aag Lys 390 | agt Ser | cga Arg | ccc Pro | cag Gln | gaa Glu 395 | aga Arg | gag Glu | gcc Ala | tcg Ser | ccc Pro 400 | 1200 |
| agc Ser | aat Asn | agc Ser | tgc Cys | ctc Leu 405 | gat Asp | tct Ser | act Thr | gac Asp | tca Ser 410 | gaa Glu | agt Ser | agc Ser | cat His | gat Asp 415 | gac Asp | 1248 |
| cgc Arg | cag Gln | tcc Ser | tac Tyr 420 | caa Gln | gga Gly | aac Asn | cct Pro | gcc Ala 425 | tta Leu | aat Asn | ccc Pro | aag Lys | agg Arg 430 | aaa Lys | caa Gln | 1296 |
| agc Ser | cca Pro | gct Ala 435 | tac Tyr | atg Met | aag Lys | gag Glu | gat Asp 440 | gtc Val | aag Lys | gct Ala | ttg Leu | gat Asp 445 | gct Ala | acc Thr | aag Lys | 1344 |
| gcc Ala | ccc Pro 450 | aag Lys | ggc Gly | tct Ser | ctg Leu | aag Lys 455 | gac Asp | atc Ile | tat Tyr | aag Lys | gtt Val 460 | ttc Phe | aat Asn | gga Gly | gaa Glu | 1392 |
| gga Gly 465 | gaa Glu | cag Gln | ata Ile | agg Arg | gcc Ala 470 | ttc Phe | aag Lys | tgt Cys | gag Glu | cac His 475 | tgc Cys | cga Arg | gtc Val | ctt Leu | ttt Phe 480 | 1440 |
| cta Leu | gac Asp | cat His | gtc Val | atg Met 485 | tac Tyr | acc Thr | att Ile | cac His | atg Met 490 | ggc Gly | tgc Cys | cat His | ggc Gly | tac Tyr 495 | cgg Arg | 1488 |
| gac Asp | cca Pro | ctg Leu | gaa Glu 500 | tgc Cys | aac Asn | atc Ile | tgt Cys | ggc Gly 505 | tac Tyr | aga Arg | agc Ser | cag Gln | gac Asp 510 | cgc Arg | tac Tyr | 1536 |
| gaa Glu | ttt Phe | tca Ser 515 | tca Ser | cac His | att Ile | gtt Val | ggg Gly 520 | ggg Gly | cag Gln | cac His | aca Thr | ttc Phe 525 | cac His | | | 1578 |
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| Ile | Asn | Asn | Ala | Ile | Thr | Tyr | Leu | Gly | Ala | Glu | Ala | Leu | His | Pro | Leu |
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| Met | Glu | Thr | Asp | Ala | Ile | Asp | Gly | Tyr | Ile | Thr | Cys | Asp | Asn | Glu | Leu | |
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| tca | ccc | gaa | ggg | gaa | cac | gcc | aat | atg | gcc | att | gac | ctc | acc | tca | agc | 96 |
| Ser | Pro | Glu | Gly | Glu | His | Ala | Asn | Met | Ala | Ile | Asp | Leu | Thr | Ser | Ser | |
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| Thr | Pro | Asn | Gly | Gln | His | Ala | Ser | Pro | Ser | His | Met | Thr | Ser | Thr | Asn | |
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| tct | gta | aag | ctg | gaa | atg | cag | agt | gat | gaa | gag | tgt | gac | agg | cag | ccc | 192 |
| Ser | Val | Lys | Leu | Glu | Met | Gln | Ser | Asp | Glu | Glu | Cys | Asp | Arg | Gln | Pro | |
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| ctg | agc | cgt | gag | gat | gag | atc | agg | ggc | cac | gat | gag | ggg | agc | agc | cta | 240 |
| Leu | Ser | Arg | Glu | Asp | Glu | Ile | Arg | Gly | His | Asp | Glu | Gly | Ser | Ser | Leu | |
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| gaa | gaa | ccc | cta | att | gag | agc | agc | gag | gtg | gcc | gac | aac | agg | aaa | gtc | 288 |
| Glu | Glu | Pro | Leu | Ile | Glu | Ser | Ser | Glu | Val | Ala | Asp | Asn | Arg | Lys | Val | |
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| cag | gac | ctt | caa | ggc | gag | gga | gga | atc | cgg | ctt | ccg | aat | ggt | gag | cgg | 336 |
| Gln | Asp | Leu | Gln | Gly | Glu | Gly | Gly | Ile | Arg | Leu | Pro | Asn | Gly | Glu | Arg | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| ccc | ttc | cac | tgt | aac | cag | tgc | gga | gct | tct | ttt | acc | cag | aag | ggc | aac | 384 |
| Pro | Phe | His | Cys | Asn | Gln | Cys | Gly | Ala | Ser | Phe | Thr | Gln | Lys | Gly | Asn | |
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| ctt | ctg | aga | cac | ata | aag | tta | cac | tct | gga | gag | aag | ccc | ttc | aaa | tgt | 432 |
| Leu | Leu | Arg | His | Ile | Lys | Leu | His | Ser | Gly | Glu | Lys | Pro | Phe | Lys | Cys | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| cct | ttc | tgt | agc | tat | gct | tgt | aga | aga | agg | gac | gct | ctc | aca | gga | cac | 480 |
| Pro | Phe | Cys | Ser | Tyr | Ala | Cys | Arg | Arg | Arg | Asp | Ala | Leu | Thr | Gly | His | |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| ctc | agg | acc | cat | tct | gtg | ggt | aaa | cct | cac | aag | tgt | aac | tac | tgt | ggc | 528 |
| Leu | Arg | Thr | His | Ser | Val | Gly | Lys | Pro | His | Lys | Cys | Asn | Tyr | Cys | Gly | |
| | | | | 165 | | | | 170 | | | | | | 175 | | |
| cga | agc | tac | aag | cag | cgc | acg | tca | ctg | gag | gaa | cac | aag | gaa | cgc | tgt | 576 |
| Arg | Ser | Tyr | Lys | Gln | Arg | Thr | Ser | Leu | Glu | Glu | His | Lys | Glu | Arg | Cys | |
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| cac | aac | tat | ctc | cag | aat | gtc | agc | atg | gag | gct | gcc | ggg | cag | gtc | atg | 624 |
| His | Asn | Tyr | Leu | Gln | Asn | Val | Ser | Met | Glu | Ala | Ala | Gly | Gln | Val | Met | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |
| agt | cac | cat | gta | ccg | cct | atg | gaa | gat | tgt | aag | gaa | caa | gag | cct | atc | 672 |
| Ser | His | His | Val | Pro | Pro | Met | Glu | Asp | Cys | Lys | Glu | Gln | Glu | Pro | Ile | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| atg | gac | aac | aat | att | tct | ctg | gtg | cct | ttt | gag | aga | cct | gct | gtc | ata | 720 |
| Met | Asp | Asn | Asn | Ile | Ser | Leu | Val | Pro | Phe | Glu | Arg | Pro | Ala | Val | Ile | |
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| | |
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| Glu Lys Leu Thr Ala Asn Met Gly Lys Arg Lys Ser Ser Thr Pro Gln | |
| 245 250 255 | |
| aag ttt gtg ggg gaa aag ctt atg cga ttc agc tac cca gat att cat | 816 |
| Lys Phe Val Gly Glu Lys Leu Met Arg Phe Ser Tyr Pro Asp Ile His | |
| 260 265 270 | |
| ttt gat atg aac tta aca tat gag aag gag gct gag ctg atg cag tct | 864 |
| Phe Asp Met Asn Leu Thr Tyr Glu Lys Glu Ala Glu Leu Met Gln Ser | |
| 275 280 285 | |
| cat atg atg gac caa gcc atc aac aat gca atc acc tac ctt gga gct | 912 |
| His Met Met Asp Gln Ala Ile Asn Asn Ala Ile Thr Tyr Leu Gly Ala | |
| 290 295 300 | |
| gag gcc ctt cac cct ctg atg cag cat gca cca agc aca atc gct gag | 960 |
| Glu Ala Leu His Pro Leu Met Gln His Ala Pro Ser Thr Ile Ala Glu | |
| 305 310 315 320 | |
| gtg gcc cca gtt ata agc tca gct tat tct cag gtc tat cat cca aac | 1008 |
| Val Ala Pro Val Ile Ser Ser Ala Tyr Ser Gln Val Tyr His Pro Asn | |
| 325 330 335 | |
| agg ata gaa aga ccc att agc agg gaa aca tct gat agt cac gaa aac | 1056 |
| Arg Ile Glu Arg Pro Ile Ser Arg Glu Thr Ser Asp Ser His Glu Asn | |
| 340 345 350 | |
| aac atg gat ggc ccc atc tct ctc atc aga cca aag agt cga ccc cag | 1104 |
| Asn Met Asp Gly Pro Ile Ser Leu Ile Arg Pro Lys Ser Arg Pro Gln | |
| 355 360 365 | |
| gaa aga gag gcc tcg ccc agc aat agc tgc ctc gat tct act gac tca | 1152 |
| Glu Arg Glu Ala Ser Pro Ser Asn Ser Cys Leu Asp Ser Thr Asp Ser | |
| 370 375 380 | |
| gaa agt agc cat gat gac cgc cag tcc tac caa gga aac cct gcc tta | 1200 |
| Glu Ser Ser His Asp Asp Arg Gln Ser Tyr Gln Gly Asn Pro Ala Leu | |
| 385 390 395 400 | |
| aat ccc aag agg aaa caa agc cca gct tac atg aag gag gat gtc aag | 1248 |
| Asn Pro Lys Arg Lys Gln Ser Pro Ala Tyr Met Lys Glu Asp Val Lys | |
| 405 410 415 | |
| gct ttg gat gct acc aag gcc ccc aag ggc tct ctg aag gac atc tat | 1296 |
| Ala Leu Asp Ala Thr Lys Ala Pro Lys Gly Ser Leu Lys Asp Ile Tyr | |
| 420 425 430 | |
| aag gtt ttc aat gga gaa gga gaa cag ata agg gcc ttc aag tgt gag | 1344 |
| Lys Val Phe Asn Gly Glu Gly Glu Gln Ile Arg Ala Phe Lys Cys Glu | |
| 435 440 445 | |
| cac tgc cga gtc ctt ttt cta gac cat gtc atg tac acc att cac atg | 1392 |
| His Cys Arg Val Leu Phe Leu Asp His Val Met Tyr Thr Ile His Met | |
| 450 455 460 | |
| ggt tgc cat ggc tac cgg gac cca ctg gaa tgc aac atc tgt ggc tac | 1440 |
| Gly Cys His Gly Tyr Arg Asp Pro Leu Glu Cys Asn Ile Cys Gly Tyr | |
| 465 470 475 480 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
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| | | | | 485 | | | | | 490 | | | | | 495 | | |

| | | | | | | |
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| | | | 20 | | | | | 25 | | | | | 30 | | |
| Thr | Pro | Asn | Gly | Gln | His | Ala | Ser | Pro | Ser | His | Met | Thr | Ser | Thr | Asn |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ser | Val | Lys | Leu | Glu | Met | Gln | Ser | Asp | Glu | Glu | Cys | Asp | Arg | Gln | Pro |
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| Leu | Ser | Arg | Glu | Asp | Glu | Ile | Arg | Gly | His | Asp | Glu | Gly | Ser | Ser | Leu |
| 65 | | | | | 70 | | | | 75 | | | | | | 80 |
| Glu | Glu | Pro | Leu | Ile | Glu | Ser | Ser | Glu | Val | Ala | Asp | Asn | Arg | Lys | Val |
| | | | | 85 | | | | 90 | | | | | | 95 | |
| Gln | Asp | Leu | Gln | Gly | Glu | Gly | Gly | Ile | Arg | Leu | Pro | Asn | Gly | Glu | Arg |
| | | | 100 | | | | 105 | | | | | | 110 | | |
| Pro | Phe | His | Cys | Asn | Gln | Cys | Gly | Ala | Ser | Phe | Thr | Gln | Lys | Gly | Asn |
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| Leu | Leu | Arg | His | Ile | Lys | Leu | His | Ser | Gly | Glu | Lys | Pro | Phe | Lys | Cys |
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| Leu | Arg | Thr | His | Ser | Val | Gly | Lys | Pro | His | Lys | Cys | Asn | Tyr | Cys | Gly |
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| Arg | Ser | Tyr | Lys | Gln | Arg | Thr | Ser | Leu | Glu | Glu | His | Lys | Glu | Arg | Cys |
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| His | Asn | Tyr | Leu | Gln | Asn | Val | Ser | Met | Glu | Ala | Ala | Gly | Gln | Val | Met |
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| Met | Asp | Asn | Asn | Ile | Ser | Leu | Val | Pro | Phe | Glu | Arg | Pro | Ala | Val | Ile |
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| Glu | Lys | Leu | Thr | Ala | Asn | Met | Gly | Lys | Arg | Lys | Ser | Ser | Thr | Pro | Gln |
| | | | | 245 | | | | 250 | | | | | | 255 | |
| Lys | Phe | Val | Gly | Glu | Lys | Leu | Met | Arg | Phe | Ser | Tyr | Pro | Asp | Ile | His |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Phe | Asp | Met | Asn | Leu | Thr | Tyr | Glu | Lys | Glu | Ala | Glu | Leu | Met | Gln | Ser |
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| Val | Ala | Pro | Val | Ile | Ser | Ser | Ala | Tyr | Ser | Gln | Val | Tyr | His | Pro | Asn |
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| | | | | | | | | | | | | | | | |
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| Glu | Arg | Glu | Ala | Ser | Pro | Ser | Asn | Ser | Cys | Leu | Asp | Ser | Thr | Asp | Ser |
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| Glu | Ser | Ser | His | Asp | Asp | Arg | Gln | Ser | Tyr | Gln | Gly | Asn | Pro | Ala | Leu |
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| Asn | Pro | Lys | Arg | Lys | Gln | Ser | Pro | Ala | Tyr | Met | Lys | Glu | Asp | Val | Lys |
| | | | | 405 | | | | | 410 | | | | | 415 | |
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| Lys | Val | Phe | Asn | Gly | Glu | Gly | Glu | Gln | Ile | Arg | Ala | Phe | Lys | Cys | Glu |
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| His | Cys | Arg | Val | Leu | Phe | Leu | Asp | His | Val | Met | Tyr | Thr | Ile | His | Met |
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| Gly | Cys | His | Gly | Tyr | Arg | Asp | Pro | Leu | Glu | Cys | Asn | Ile | Cys | Gly | Tyr |
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| Arg | Ser | Gln | Asp | Arg | Tyr | Glu | Phe | Ser | Ser | His | Ile | Val | Gly | Gly | Gln |
| | | | 485 | | | | | | 490 | | | | | 495 | |
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<220>
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| cgacagagct ggaaatggtg atgaatcttt tttaatcaaa ggacaatttc ttttcattgc | 180 |
| actttgact atg gaa aca gag gct att gat ggc tat ata acg tgt gac aat | 231 |
| Met Glu Thr Glu Ala Ile Asp Gly Tyr Ile Thr Cys Asp Asn | |
| 1 5 10 | |
| | |
| gag ctt tca ccc gaa agg gag cac tcc aat atg gca att gac ctc acc | 279 |
| Glu Leu Ser Pro Glu Arg Glu His Ser Asn Met Ala Ile Asp Leu Thr | |
| 15 20 25 30 | |
| | |
| tca agc aca ccc aat gga cag cat gcc tca cca agt cac atg aca agc | 327 |
| Ser Ser Thr Pro Asn Gly Gln His Ala Ser Pro Ser His Met Thr Ser | |
| 35 40 45 | |
| | |
| aca gat tca gta aag cta gaa atg cag agt gat gaa gag tgt gac agg | 375 |
| Thr Asp Ser Val Lys Leu Glu Met Gln Ser Asp Glu Glu Cys Asp Arg | |
| 50 55 60 | |
| | |
| aaa ccc ctg agc cgt gaa gat gag atc agg ggc cat gat gag ggt agc | 423 |
| Lys Pro Leu Ser Arg Glu Asp Glu Ile Arg Gly His Asp Glu Gly Ser | |
| 65 70 75 | |
| | |
| agc cta gaa gaa ccc cta att gag agc agc gag gtg gct gac aac agg | 471 |
| Ser Leu Glu Glu Pro Leu Ile Glu Ser Ser Glu Val Ala Asp Asn Arg | |
| 80 85 90 | |
| | |
| gaa gtc cag gag ctt caa ggc gag gga gga atc cgg ctt ccg aat ggt | 519 |
| Glu Val Gln Glu Leu Gln Gly Glu Gly Gly Ile Arg Leu Pro Asn Gly | |
| 95 100 105 110 | |

| | |
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| aaa ctg aaa tgt gac gtc tgt ggc atg gtt tgc att ggg ccc aat gtg | 567 |
| Lys Leu Lys Cys Asp Val Cys Gly Met Val Cys Ile Gly Pro Asn Val | |
| 115 120 125 | |
| ctt atg gta cat aaa agg agt cac act ggt gaa cgc ccc ttc cac tgt | 615 |
| Leu Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro Phe His Cys | |
| 130 135 140 | |
| aac cag tgt gga gct tct ttt act cag aag ggc aac ctt ctg aga cac | 663 |
| Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His | |
| 145 150 155 | |
| ata aag tta cac tct gga gag aag ccg ttc aaa tgt cct ttc tgt agt | 711 |
| Ile Lys Leu His Ser Gly Glu Lys Pro Phe Lys Cys Pro Phe Cys Ser | |
| 160 165 170 | |
| cac gcc tgt aga aga agg gac gcc ctc aca gga tac ctc agg acc cat | 759 |
| His Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly Tyr Leu Arg Thr His | |
| 175 180 185 190 | |
| tct gtg ggt aaa cct cac aag tgc aac tac tgt gga cga agc tac aag | 807 |
| Ser Val Gly Lys Pro His Lys Cys Asn Tyr Cys Gly Arg Ser Tyr Lys | |
| 195 200 205 | |
| cag cgc agt tca ctg gag gag cac aag gaa cgc tgc cac aac tat ctc | 855 |
| Gln Arg Ser Ser Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu | |
| 210 215 220 | |
| cag aat gtc agc atg gag gct gct ggg cag gtc atg agt cac cat gta | 903 |
| Gln Asn Val Ser Met Glu Ala Ala Gly Gln Val Met Ser His His Val | |
| 225 230 235 | |
| cct cct atg gaa gat tgt aag gaa caa gag cct att atg gac aac aat | 951 |
| Pro Pro Met Glu Asp Cys Lys Glu Gln Glu Pro Ile Met Asp Asn Asn | |
| 240 245 250 | |
| att tct ctg gtg cct ttt gag aga cct gct gtc ata gag aag ctc acg | 999 |
| Ile Ser Leu Val Pro Phe Glu Arg Pro Ala Val Ile Glu Lys Leu Thr | |
| 255 260 265 270 | |
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| Gly Asn Met Gly Lys Arg Lys Ser Ser Thr Pro Gln Lys Phe Val Gly | |
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| Glu Lys Leu Met Arg Phe Ser Tyr Pro Asp Ile His Phe Asp Met Asn | |
| 290 295 300 | |
| tta aca tat gag aag gag gct gag ctg atg cag tct cat atg atg gac | 1143 |
| Leu Thr Tyr Glu Lys Glu Ala Glu Leu Met Gln Ser His Met Met Asp | |
| 305 310 315 | |
| caa gcc atc aac aat gca atc acc tac ctt gga gct gag gcc ctt cac | 1191 |
| Gln Ala Ile Asn Asn Ala Ile Thr Tyr Leu Gly Ala Glu Ala Leu His | |
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| cct ctg atg cag cac ccg cca agc aca atc gct gaa gtg gcc cca gtt | 1239 |
| Pro Leu Met Gln His Pro Pro Ser Thr Ile Ala Glu Val Ala Pro Val | |
| 335 340 345 350 | |

| | |
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| ata agc tca gct tat tct cag gtc tat cat cca aat agg ata gaa aga | 1287 |
| Ile Ser Ser Ala Tyr Ser Gln Val Tyr His Pro Asn Arg Ile Glu Arg | |
| 355 360 365 | |
| ccc att agc agg gaa act gct gat agt cat gaa aac aac atg gat ggc | 1335 |
| Pro Ile Ser Arg Glu Thr Ala Asp Ser His Glu Asn Asn Met Asp Gly | |
| 370 375 380 | |
| ccc atc tct ctc atc aga cca aag agt cga ccc cag gaa aga gag gcc | 1383 |
| Pro Ile Ser Leu Ile Arg Pro Lys Ser Arg Pro Gln Glu Arg Glu Ala | |
| 385 390 395 | |
| tct ccc agc aat agc tgc ctg gat tcc act gac tca gaa agc agc cat | 1431 |
| Ser Pro Ser Asn Ser Cys Leu Asp Ser Thr Asp Ser Glu Ser Ser His | |
| 400 405 410 | |
| gat gac cac cag tcc tac caa gga cac cct gcc tta aat ccc aag agg | 1479 |
| Asp Asp His Gln Ser Tyr Gln Gly His Pro Ala Leu Asn Pro Lys Arg | |
| 415 420 425 430 | |
| aaa caa agc cca gct tac atg aag gag gat gtc aaa gct ttg gat act | 1527 |
| Lys Gln Ser Pro Ala Tyr Met Lys Glu Asp Val Lys Ala Leu Asp Thr | |
| 435 440 445 | |
| acc aag gct cct aag ggc tct ctg aag gac atc tac aag gtc ttc aat | 1575 |
| Thr Lys Ala Pro Lys Gly Ser Leu Lys Asp Ile Tyr Lys Val Phe Asn | |
| 450 455 460 | |
| ggg gaa gga gaa cag att agg gcc ttc aag tgt gag cac tgc cga gtc | 1623 |
| Gly Glu Gly Glu Gln Ile Arg Ala Phe Lys Cys Glu His Cys Arg Val | |
| 465 470 475 | |
| ctt ttc cta gac cat gtc atg tac acc att cac atg ggt tgc cat ggc | 1671 |
| Leu Phe Leu Asp His Val Met Tyr Thr Ile His Met Gly Cys His Gly | |
| 480 485 490 | |
| tac cgg gac cca ctg gaa tgt aac atc tgt ggc tac aga agc cag gac | 1719 |
| Tyr Arg Asp Pro Leu Glu Cys Asn Ile Cys Gly Tyr Arg Ser Gln Asp | |
| 495 500 505 510 | |
| cgt tat gag ttt tca tca cac att gtt cga ggg gag cac aca ttc cac | 1767 |
| Arg Tyr Glu Phe Ser Ser His Ile Val Arg Gly Glu His Thr Phe His | |
| 515 520 525 | |
| taggcctttt cattccaaag gggaccctat gaagtaaaga ctgcacatga agaaatactg | 1827 |
| cacttacaat cccacctttc ctcaaagtgt gtacctttta tttttttaat ataatactgg | 1887 |
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 Ser Val Lys Leu Glu Met Gln Ser Asp Glu Glu Cys Asp Arg Lys Pro
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| Glu | Glu | Pro | Leu | Ile | Glu | Ser | Ser | Glu | Val | Ala | Asp | Asn | Arg | Glu | Val |
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| Gln | Glu | Leu | Gln | Gly | Glu | Gly | Gly | Ile | Arg | Leu | Pro | Asn | Gly | Lys | Leu |
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| Val | His | Lys | Arg | Ser | His | Thr | Gly | Glu | Arg | Pro | Phe | His | Cys | Asn | Gln |
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| Cys | Gly | Ala | Ser | Phe | Thr | Gln | Lys | Gly | Asn | Leu | Leu | Arg | His | Ile | Lys |
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| Leu | His | Ser | Gly | Glu | Lys | Pro | Phe | Lys | Cys | Pro | Phe | Cys | Ser | His | Ala |
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| Cys | Arg | Arg | Arg | Asp | Ala | Leu | Thr | Gly | Tyr | Leu | Arg | Thr | His | Ser | Val |
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| Gly | Lys | Pro | His | Lys | Cys | Asn | Tyr | Cys | Gly | Arg | Ser | Tyr | Lys | Gln | Arg |
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| | 210 | | | | | 215 | | | | | 220 | | | | |
| Val | Ser | Met | Glu | Ala | Ala | Gly | Gln | Val | Met | Ser | His | His | Val | Pro | Pro |
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| Met | Glu | Asp | Cys | Lys | Glu | Gln | Glu | Pro | Ile | Met | Asp | Asn | Asn | Ile | Ser |
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| Leu | Val | Pro | Phe | Glu | Arg | Pro | Ala | Val | Ile | Glu | Lys | Leu | Thr | Gly | Asn |
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| Tyr | Glu | Lys | Glu | Ala | Glu | Leu | Met | Gln | Ser | His | Met | Met | Asp | Gln | Ala |
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| Ile | Asn | Asn | Ala | Ile | Thr | Tyr | Leu | Gly | Ala | Glu | Ala | Leu | His | Pro | Leu |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Met | Gln | His | Pro | Pro | Ser | Thr | Ile | Ala | Glu | Val | Ala | Pro | Val | Ile | Ser |
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| Ser | Ala | Tyr | Ser | Gln | Val | Tyr | His | Pro | Asn | Arg | Ile | Glu | Arg | Pro | Ile |
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| Ser | Arg | Glu | Thr | Ala | Asp | Ser | His | Glu | Asn | Asn | Met | Asp | Gly | Pro | Ile |
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| Ser | Leu | Ile | Arg | Pro | Lys | Ser | Arg | Pro | Gln | Glu | Arg | Glu | Ala | Ser | Pro |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Ser | Asn | Ser | Cys | Leu | Asp | Ser | Thr | Asp | Ser | Glu | Ser | Ser | His | Asp | Asp |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| His | Gln | Ser | Tyr | Gln | Gly | His | Pro | Ala | Leu | Asn | Pro | Lys | Arg | Lys | Gln |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Ser | Pro | Ala | Tyr | Met | Lys | Glu | Asp | Val | Lys | Ala | Leu | Asp | Thr | Thr | Lys |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Ala | Pro | Lys | Gly | Ser | Leu | Lys | Asp | Ile | Tyr | Lys | Val | Phe | Asn | Gly | Glu |
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| Gly | Glu | Gln | Ile | Arg | Ala | Phe | Lys | Cys | Glu | His | Cys | Arg | Val | Leu | Phe |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Leu | Asp | His | Val | Met | Tyr | Thr | Ile | His | Met | Gly | Cys | His | Gly | Tyr | Arg |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Asp | Pro | Leu | Glu | Cys | Asn | Ile | Cys | Gly | Tyr | Arg | Ser | Gln | Asp | Arg | Tyr |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Glu | Phe | Ser | Ser | His | Ile | Val | Arg | Gly | Glu | His | Thr | Phe | His | | |
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 Ser Asp Arg Gly Met Gly Ser Asn Val Lys Val Glu Thr Gln Ser Asp
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 Glu Glu Asn Gly Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu
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 Asp Leu Arg Met Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His
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 Arg Asp Gln Gly Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu
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 Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Val Cys Ile Gly
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 Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Glu Arg Pro Phe
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 Gln Cys Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu
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 Arg His Ile Lys Leu His Ser Gly Glu Lys Pro Phe Lys Cys His Leu
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 Cys Asn Tyr Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly His Leu Arg
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 Thr His Ser Val Gly Lys Pro His Lys Cys Gly Tyr Cys Gly Arg Ser
 195 200 205
 Tyr Lys Gln Arg Ser Ser Leu Glu Glu His Lys Glu Arg Cys His Asn
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 Tyr Leu Glu Ser Met Gly Leu Pro Gly Val Cys Pro Val Ile Lys Glu
 225 230 235 240
 Glu Thr Asn His Asn Glu Met Ala Glu Asp Leu Cys Lys Ile Gly Ala
 245 250 255
 Glu Arg Ser Leu Val Leu Asp Arg Leu Ala Ser Asn Val Ala Lys Arg
 260 265 270
 Lys Ser Ser Met Pro Gln Lys Phe Leu Gly Asp Lys Cys Leu Ser Asp
 275 280 285
 Met Pro Tyr Asp Ser Ala Asn Tyr Glu Lys Glu Asp Met Met Thr Ser
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 His Val Met Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala
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 Glu Ser Leu Arg Pro Leu Val Gln Thr Pro Pro Gly Ser Ser Glu Val
 325 330 335
 Val Pro Val Ile Ser Ser Met Tyr Gln Leu His Lys Pro Pro Ser Asp
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 Gly Pro Pro Arg Ser Asn His Ser Ala Gln Asp Ala Val Asp Asn Leu
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 Leu Leu Leu Ser Lys Ala Lys Ser Val Ser Ser Glu Arg Glu Ala Ser
 370 375 380
 Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser Asn Ala Glu
 385 390 395 400
 Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr Asn His Ile Asn Pro His
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 Ala Arg Asn Gly Leu Ala Leu Lys Glu Glu Gln Arg Ala Tyr Glu Val
 420 425 430
 Leu Arg Ala Ala Ser Glu Asn Ser Gln Asp Ala Phe Arg Val Val Ser
 435 440 445
 Thr Ser Gly Glu Gln Leu Lys Val Tyr Lys Cys Glu His Cys Arg Val
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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Phe | Leu | Asp | His | Val | Met | Tyr | Thr | Ile | His | Met | Gly | Cys | His | Gly |
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| Cys | His | Gly | Phe | Arg | Asp | Pro | Phe | Glu | Cys | Asn | Met | Cys | Gly | Tyr | His |
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<210> 8
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| Glu | Glu | Ser | Glu | Met | Pro | Tyr | Ser | Tyr | Ala | Arg | Glu | Tyr | Ser | Asp | Tyr |
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| Glu | Ser | Ile | Lys | Leu | Glu | Arg | His | Val | Pro | Tyr | Asp | Asn | Ser | Arg | Pro |
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| Thr | Ser | Gly | Lys | Met | Asn | Cys | Asp | Val | Cys | Gly | Leu | Ser | Cys | Ile | Ser |
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| Phe | Asn | Val | Leu | Met | Val | His | Lys | Arg | Ser | His | Thr | Gly | Glu | Arg | Pro |
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| Phe | Gln | Cys | Asn | Gln | Cys | Gly | Ala | Ser | Phe | Thr | Gln | Lys | Gly | Asn | Leu |
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| Leu | Arg | His | Ile | Lys | Leu | His | Thr | Gly | Glu | Lys | Pro | Phe | Lys | Cys | His |
| | | | 165 | | | | | 170 | | | | | | 175 | |
| Leu | Cys | Asn | Tyr | Ala | Cys | Gln | Arg | Arg | Asp | Ala | Leu | Thr | Gly | His | Leu |
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| Arg | Thr | His | Ser | Val | Glu | Lys | Pro | Tyr | Lys | Cys | Glu | Phe | Cys | Gly | Arg |
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| Ser | Tyr | Lys | Gln | Arg | Ser | Ser | Leu | Glu | Glu | His | Lys | Glu | Arg | Cys | Arg |
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| Arg | His | Ile | Lys | Ala | Glu | Met | Gly | Ser | Glu | Arg | Ala | Leu | Val | Leu | Asp |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Arg | Leu | Ala | Ser | Asn | Val | Ala | Lys | Arg | Lys | Ser | Ser | Met | Pro | Gln | Lys |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Phe | Ile | Gly | Glu | Lys | Arg | His | Cys | Phe | Asp | Ala | Asn | Tyr | Asn | Pro | Gly |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Tyr | Met | Tyr | Glu | Lys | Glu | Asn | Glu | Met | Met | Gln | Thr | Arg | Met | Met | Asp |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Gln | Ala | Ile | Asn | Asn | Ala | Ile | Ser | Tyr | Leu | Gly | Ala | Glu | Ala | Phe | Arg |
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| Pro | Leu | Val | Gln | Thr | Pro | Pro | Ala | Pro | Thr | Ser | Glu | Met | Val | Pro | Val |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Ile | Ser | Ser | Val | Tyr | Pro | Ile | Ala | Leu | Thr | Arg | Ala | Asp | Met | Pro | Met |
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| Gly | Ala | Pro | Gln | Glu | Met | Glu | Lys | Lys | Arg | Ile | Leu | Leu | Pro | Glu | Lys |
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| Gln | Ser | His | Val | Val | Leu | Pro | Gln | Ala | Arg | Asn | Gly | Met | Pro | Leu | Leu |
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| Lys | Glu | Val | Pro | Arg | Ser | Phe | Glu | Leu | Leu | Lys | Pro | Pro | Pro | Ile | Cys |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Leu | Arg | Asp | Ser | Ile | Lys | Val | Ile | Asn | Lys | Glu | Gly | Glu | Val | Met | Asp |
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| Val | Phe | Arg | Cys | Asp | His | Cys | His | Val | Leu | Phe | Leu | Asp | Tyr | Val | Met |
| | 450 | | | | | 455 | | | | | 460 | | | | |
| Phe | Thr | Ile | His | Met | Gly | Cys | His | Gly | Phe | Arg | Asp | Pro | Phe | Glu | Cys |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Asn | Met | Cys | Gly | Tyr | Arg | Ser | His | Asp | Arg | Tyr | Glu | Phe | Ser | Ser | His |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Ile | Ala | Arg | Gly | Glu | His | Arg | Ala | Met | Leu | Lys | | | | | |
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33

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| <400> 17 | 20 |
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COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled THE HELIOS GENE, the specification of which

☒ is attached hereto.

☐ was filed on _____ as Application Serial No. _____
and was amended on _____.

☐ was described and claimed in PCT International Application No. _____
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

| U.S. SERIAL NO. | FILING DATE | STATUS |
|-------------------|--------------------------|----------------|
| <u>60/076,325</u> | <u>February 27, 1998</u> | <u>PENDING</u> |

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Louis Myers, Reg. No. 35,965

Address all telephone calls to Louis Myers, Esq. at telephone number 617/542-5070.

Address all correspondence to Louis Myers, Esq., Fish & Richardson P.C., 225 Franklin Street ,
Boston, MA 02110-2804.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: Katia Georgopoulos

Inventor's Signature: _____ Date: _____

Residence Address: 28 R.C. Kelley Street, Cambridge, MA 02138

Citizen of: USA

Post Office Address: 28 R.C. Kelley Street, Cambridge, MA 02138

COMBINED DECLARATION AND POWER OF ATTORNEY CONTINUED

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